UNIVERSITAT ROVIRA I VIRGILI

EXTRACTION AND VALORIZATION OF CELLULOSE FROM MUNICIPAL AND INDUSTRIAL WASTE BIOMASS

Katarzyna Glińska

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Department of Chemical Engineering

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Supervised by Dr. Christophe Bengoa

Chemical Engineering Department

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I STATE that the present study, entitled "Extraction and valorization of cellulose from municipal and industrial waste biomass", presented by Katarzyna Glińska for the award of the degree of Doctor, has been carried out under my supervision at the Department of Chemical Engineering of this university.

Tarragona, 10th June 2020

Dr. Christophe Bengoa
Doctoral Thesis Supervisor

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Table of Contents

Summary

Cellulose is the most abundant organic polymer on the planet. Due to its properties, such as biocompatibility, biodegradability, thermal and chemical stability has been used in many applications. Cellulose can be obtained from biomass waste without exposing the food and feed supplies, forests, and biodiversity in the world. This thesis investigates the valorisation of lignocellulosic biomass waste: industrial paper sludge, municipal sewage sludge and agricultural corn stover as a potential source of carbohydrates, mainly cellulose, which can be converted into value-added molecules. At the same time, this work follows the concept of circular economy for biomass waste.

Paper industry generates large amounts of dried sludge (40 and 50 kg) per ton of paper produced. The cellulose contained in the residual sludge can be reused or converted into other materials. The first study realised in this thesis evaluates the influence of commercial and cheap phosphonium-based ionic liquid on cellulose separation from industrial paper mill wastewater dried sludge. The ionic liquid demonstrated potential for cellulose recovery from industrial paper sludge as well as ability to dissolve ashes from paper sludge.

Additional study realised on industrial paper mill wastewater dried sludge aimed at characterization of paper sludge by standard methods; cellulose recovery by phosphonium and imidazolium based ionic liquids, and characterization of recovered cellulose. As a result of this study, both ionic liquids were able to recover the cellulose of the sludge (~100%). The ratio sludge: ionic liquid had only a low influence on the recovery. Phosphonium ionic liquid was able to clean better the cellulose, eliminating a major quantity of ashes. Imidazolium ionic liquid was able to leave a minor quantity of initial proteins in the cellulose. However, improvement of the performances of the ionic liquids is necessary to allow a scale-up of the technology.

Since the recovery of cellulose from industrial paper sludge was feasible with ionic liquids, the optimization study about cellulose recovery was realised from the other type of sludge, municipal primary sewage sludge. The influence on amounts of carbohydrates, proteins and ashes was investigated based on three independent factors: temperature of dissolution; time of dissolution and sludge: ionic liquid ratio. The ionic liquid, tetrakis (hydroxymethyl) phosphonium chloride, $[P(CH_2OH)_4]Cl$, allowed the recovery of all cellulose, although it was not able to completely clean the cellulose. 36% of ashes and 51% of the proteins remained in the recovered cellulose. The experimental design did not manage to optimize the operative conditions of the process. All examined conditions were able to recover maximal values of cellulose. Experiments realised at 60℃ for 13 h and with ratio of dry sludge: ionic liquid 1 g:12 ml allowed the recovery of high quantities of cellulose, the best removal of ashes but still maintaining high amounts of proteins in the recovered cellulose.

In recent years the dissolution of lignocellulosic biomass in ionic liquid has drawn a great attention. Ionic liquids are used as green replacement for harmful volatile organic solvents due to their non-volatile character, excellent chemical and thermal stability. One classical example of lignocellulosic biomass is corn stover, which basically consists of cellulose, hemicellulose, lignin and ash. The use of designed ionic liquids with improved reusing capabilities was evaluated to recover the cellulose from corn stover. The ionic liquids were synthesized during the research stay at QUILL group (Queen's University Ionic Liquid Laboratories) in Belfast. Two imidazolium-based ionic liquids, able to bring hydrophobic and hydrophilic (hexane/water) solvents together as stable microemulsions and three phosphonium-based ionic liquids were evaluated for the recovery of cellulose. The experiments were carried out under mild conditions. The ionic liquids showed the ability to break cell walls and extract cellulose from the corn stover. Tetrabutylphosphonium 2-ethylhexanoate [P_{4444}][EH] was the ionic liquid able to extract the highest quantity of the available cellulose (84%) while imidazolium-based ionic liquids can be considered as easily recovered due to their abilities to form microemulsions and being separated.

Finally, the thesis also investigates the valorisation of cellulose recovered from WWTP sludge to added value levulinic acid with a Brønsted acidic ionic liquid. The recovered the cellulose from the urban and industrial sludge was bleached to decrease the content of ash and proteins still presented. Then, the bleached cellulose was used to produce levulinic acid through a hydrothermal catalysed process. The results demonstrate that both types of sludge can be used as precursors to obtain value-added chemicals. However, direct conversion of the sludge gave very low yield of levulinic acid. This suggests that the recovery of cellulose is an essential step in production of value-added molecules.

Chapter 1

Introduction

1. Earth Overshoot Day

Earth Overshoot Day is the day in the year when all world population have depleted the natural resources that Earth can offer that year. According to Global Footprint Network, over the past 50 years humanity's annual demand on nature exceeds what Earth's ecosystems can regenerate. In 2019, as it can be seen in Figure 1, it has moved up to July 29, the earliest ever.

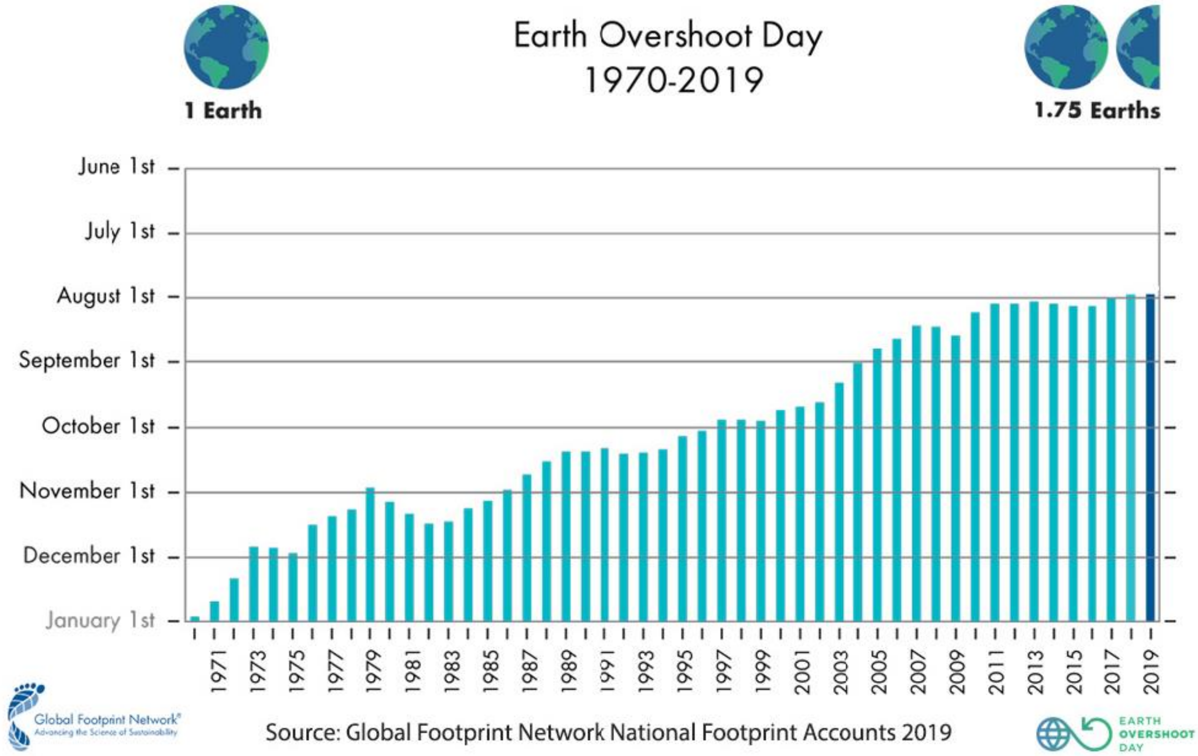


Figure 1. Earth Overshoot Day since 1970 to 2019 (Global Footprint Network https://www.overshootday.org/newsroom/past-earth-overshoot-days/, accessed 2019).

In 2008 and between the years 2014 and 2016 that day was slightly delayed due to economic crisis. The economic stagnation had impact on the work of industries reducing demand on natural resources. However, currently humanity is using nature 1.75 times faster than our planet's ecosystem can regenerate, which is equivalent to 1.75 Earths (Global Footprint Network). Given this situation, it is vital to develop solutions which move towards the circular economy, turning waste into resources and achieving among all to postpone this date (Smol et al., 2017; Hobson 2019).

2. The concept of circular economy

Due to depletion of natural resources and increasing greenhouse emission, a worldwide interest in the utilization of biomass instead fossil resources for a range of applications is growing. In

2011, nearly 15 billion tonnes of biomass were used for food, fodder and processing (sugar, starch, vegetable oils and others), whilst the energy industry consumed 2.1 billion tonnes (Scarlat et al., 2015). By 2050, the objective is that 55 % of all energy consumed in the European Union will be sourced renewably (European Commission 2011). Therefore, this will rise biomass utilisation by its incineration to produce bioenergy and consequently it will cause a sizeable increase in the amount of biomass waste (Medina et al., 2017). In the context of circular economy, the economic and environmental costs of such practices are unacceptable. Hence the research focus on valorisation of this waste based on European Union Directive 2008/98/EC (2008/98/EC) on waste and the 3Rs principle: waste 'reduction, reuse and recycling'. Plastics, food waste, critical raw materials, construction and demolition waste, biomass and bio-based products were identified as materials towards a circular economy (EC 2015).

3. Waste biomass

Biomass waste is a feedstock that can be obtained from a wide array of sources without exposing the food and feed supplies, forests, and biodiversity in the world. European Commission in 2011 declared that "if waste is to become a resource to be fed back into the economy as a raw material, then, much higher priority needs to be given to reuse and recycling" (European Commission 2011 COM). The concept of "zero waste economy" encourages utilization of waste biomass as raw materials, in substitution of fossil resources, to be directly reused and modified to new molecules or to produce biofuels.

There are 3 major types of biomass waste:

- Agricultural residue
- Forestry residue
- Industrial waste from: wood industry, food industry, pulp and paper industry and municipal solid waste and sewage.

The worldwide production of agricultural residue is estimated at about 998 million tonnes annually (Obi et al., 2016). Agricultural wastes are divided in four main groups: crop residues (bagasse, straw, stem, stalk, leaves, husk, shell, peel, pulp, stubble, etc); animal waste (excreta, carcasses); processing waste (packaging material) and hazardous waste (pesticides, insecticides and herbicides) (Nagendran 2011). The technology of utilization of agriculture waste must comply 2 requirements: a rapid use of the residues and/or storage under conditions that do not cause rotting. Commonly used agricultural waste is corn stover. Its global production is around 1 billion tons (Ruan et al., 2019). Corn stover is composed of three main polymers which are: cellulose (~35 % w/w), hemicellulose (~20 % w/w) and lignin (~12% w/w). Due to its composition can be used as fertilizers, methane producers, adsorbents in the elimination of heavy metals, animal feed, fuels by direct combustion (Obi et al., 2016), ethanol (Taghizadeh-Alisaraei et al., 2019) and bioblocks producers (Badgujar et al., 2019).

In the case of the forest waste, there are two types of residues: primary and secondary. The primary is generated by operations such as thinning of plantations, clearing for logging roads and natural attrition. The secondary, which is also treated as industrial waste, consists of by-products and co-products of industrial wood processing operation as bark, sawmill slabs, saw dust, wood chips etc. (European Commission 2018).

Industrial waste represents the biggest quantity of biomass waste generated by human activity. Wood industry produces residues from sawmilling, plywood, wood panel, furniture, building component, flooring, particle board, moulding, jointing and craft industries. The amount of waste generated from wood processing industries varies from one type industry to another depending on the form of raw material and finished product (European Commission 2018). Pulp and paper industry is other example of highly polluting industry which consumes large amount of energy and water in various operations (Buruberri et al., 2015). In 2015, the world production of paper was 400 million tons and in 2017, the European production of paper was 83 million tons (Faubert et al., 2016). Based on the data of European Paper Recycling Council, 72.3% of all paper consumed in Europe in 2017 was recycled. Paper recycling requires prior cleaning. Hence, during the cleaning average paper industry simultaneously generates 150 kg of dry solids by ton of produced paper (Deviatkin et al., 2016) and huge volumes of wastewater (Adhikari and Bhattacharyya, 2015). For these reasons, a major concern of paper industry is management of the residues. One of adopted solutions, which deal with that problem, is incineration for energy recovery. Nevertheless, this resolution is associated with a high economic cost due to high-water content and emission of greenhouse gases (Faubert et al., 2016). Other ones, are disposal in agriculture as fertilizer, production of bioethanol or production of new materials (Farghaly et al., 2017).

On the other hand, the food industry produces a large number of residues and by-products that can be used as biomass energy sources and to produce new molecules. Global food waste has been estimated to be 1.6 billion tons (Lee et al., 2020). These waste materials are generated from the portions of food from plant and animals, which contain small nutritional value or inedible parts.

As a last example of biomass waste coming from industry is sewage sludge. Sewage sludge is an inevitable waste formed during municipal wastewater treatment processes. This includes household waste liquid from toilets, baths, showers, kitchens, sinks, rain etc (Tyagi and Lo, 2013).

4. Sewage sludge

Municipal sewage sludge is a biologically mixture of water, ashes, organic matter and dead and alive microorganisms. Moreover, sewage sludge contains of large concentration of nitrogen and phosphorus and also includes contaminants as: heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), absorbable organohalogens (AOX), pesticides, surfactants, hormones, pharmaceuticals, nanoparticles and many others (Kacprzak et al., 2017). The quantity of wastewater sludge inevitably increases with the increase of population. The European Commission Report from 2008 says that more than 10 million tons of dry solid of sludge were produced in European Union (EU) in 2008, and that amount is expected to continue to grow up to 13 million tons by 2020 (EC, 2008).

The production of sludge cannot be avoided. The excess sludge amount needs to be disposed. Current methods are: incineration, landfilling, ocean-dumping, reuse in agriculture, use as combustible for cement, bricks and asphalt production (Anjum et al., 2016; Qian et al., 2016; Zhang et al., 2017). According to the literature (Pilli et al., 2015), sewage disposal processes are responsible for 40 % of the total greenhouse gas (GHG) emissions from WWTPs. The application of circular economy concept must decrease this percentage. Given this situation, sewage sludge has two main directions of management, organic recycling (associated with the use of fertilizer and soil formation potential of sewage sludge) and recycling of the energy and material (associated with the use of fuel properties and other minerals created in the waste after thermal transformation) (Kacprzak et al., 2017; Kwarciak-Kozłowska, 2019). In Figure 2 are presented the possibilities to obtain main resources (nutrients, heavy metals, adsorbents, construction materials, bio–plastics, proteins and enzymes), energy, and raw materials (biogas, syngas) from the sludge.

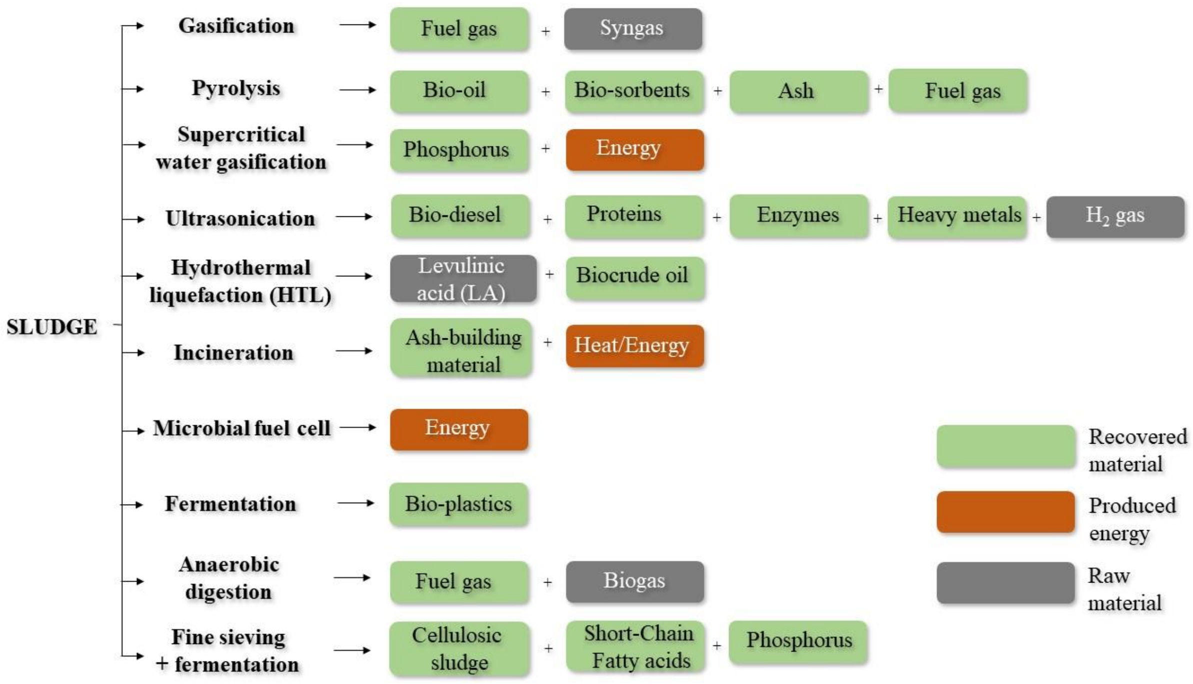


Figure 2. Produced energy, recovered and raw material from wastewater sludge adapted from (Gherghel et al., 2019).

A typical municipal WWTP produces three main types of sludge: primary, secondary and digested. Table 1 presents the characteristics of these types of sludge. The primary sludge is a combination of floating grease and solids consist of valuables organic components (protein, lipids, and carbohydrates) and inorganic ashes. Among these, carbohydrates are major components, around 26.2 ± 2.6 % based on dry matter (Olkiewicz et al., 2015a), obtained essentially from the discharge of toilet paper (Honda et al., 2002). The secondary sludge is a complex biomass, composed mainly of microbial cells and suspended solids (57.0 ± 4.2) produced during the aerobic biological treatment of primary treated wastewater and collected in the secondary settler (Tyagi and Lo, 2013). Finally, the digested sludge is formed of 60 % primary and 40 % secondary sludge. During sludge digestion organic solids are decomposed into stable substances. Digestion reduces the total mass of solids and odors, destroys pathogens and makes the sludge easier to be dewatered or dried (Pastore et al., 2013). Among all three types of sludge, the primary sludge has the greatest amount of carbohydrates and cellulose is significant part of them (Olkiewicz et al., 2015a). Recovered cellulose from the sludge could be used in packaging industry, for bioethanol production or as a substrate to obtain a value-added chemical.

Table 1. Characteristics of three types of sludge.

	Sludge		
	Primary	Secondary	Digested
Total solids (TS), %	4.2 ± 1.2 [a]	2.8 ± 0.0 [c]	3.5 ± 0.0 [c]
Volatile solids (VS), %	3.3 ± 0.2 [a]	2.4 ± 0.3 [c]	2.9 ± 0.2 [c]
Lipids, %	27.2 ± 0.4 [b]	9.0 ± 1.0 [c]	21.0 ± 1.0 [c]
Proteins, %	24.2 ± 1.4 [b]	57.0 ± 4.2 [c]	39.5 ± 0.5 [c]
Carbohydrates, %	26.2 ± 2.6 [b]	13.7 ± 0.6 [c]	22.8 ± 0.3 [c]
Ashes, %	20.1 ± 0.4 [b]	12.3 ± 0.3 [c]	17.2 ± 0.2 [c]

[a] based on literature Olkiewicz et al., 2015b

[b] based on literature Olkiewicz et al., 2015a

[c] based on characterization realized in the laboratory

5. Cellulose and classical extractions processes

5.1. Characteristic of cellulose

The most abundant organic polymer on the Earth is cellulose. Cellulose is a polysaccharide built of linear chain of hundreds to thousands of β (1 →4) linked D-glucose units. It is mainly presented in cell walls, green plants, wood (Gherghel et al., 2019; Verma et al., 2019) or sewage sludge, especially in primary sludge. Its main problem, due to a high crystallinity, is the difficulty to dissolve it in common organic solvents. The structure of cellulose is a three-dimensional arrangement of hydrogen bond (H–bond) where the hydroxyl groups are attached to each polymer chain. For this reason, a solvent has to be able to separate the polymer structure of cellulose by the disruption of the intermolecular H–bonds (Mohd et al., 2017).

Cellulose because of its main properties, such as biocompatibility, biodegradability, thermal and chemical stability has been used in many commercial applications, serving as a raw material to produce paper, pharmaceutical compounds, cosmetics, textile (Elhi et al., 2016; Mohd et al., 2017). Cellulose can be obtained on an industrial scale by conventional processes which involve extraction of cellulose by dissolving lignin that binds the cellulose fibres together. Below there are described some processes used in industry to obtain cellulose.

5.2. The Kraft process

The most dominant process to obtain lignin from the lignocellulosic biomass is the Kraft pulping procedure. In the Kraft process, aqueous sodium hydroxide along with sulphides are used to separate lignin from the cellulose under high pH condition, at a temperature range of 423–453 K, 120 psi steam pressure and for 0.5 – 3.0 h (Bajpai, 2018). After separation of cellulose from lignocellulosic biomass, lignin along with hemicellulose is collected in the form of black liquor. Post this, black liquor is concentrated in evaporators (Bonhivers and Stuart, 2013). Eventually, concentrated and lignin-rich solid is obtained that has application in boilers for steam and heat generation. Moreover, sulfur and caustic content is recycled, thereby leading to an improved economics of the process. However, lignin obtained through the kraft process possesses low heating value and emits more poisonous gases as compared with coal (Ahmad et al., 2018). Additionally, it is possible to recover the lignin obtained from kraft process by lowering the pH of the black liquor with carbon dioxide. Then, precipitated lignin can be recovered from black liquor via simple filtration and washing. In order to obtain high purity lignin, precipitated lignin is redissolved in water and acid. Next, the slurry is once again dewatered and washed (Mathew et al., 2018). Undoubtedly, kraft pulping is a well-known and established process currently used to produce the majority of lignin throughout the world (Ahmad et al., 2018).

5.3. The Organosolv process

A large number of organic solvents like acetone, methanol, ethanol, propanol, butanol, ethylene glycol, formic acid, acetic acid, amines, ethers, esters, formaldehyde are used in the Organosolv process. The solvents can work with or without catalyst in the temperature range of 373–473K. The concentration of solvent in water ranges from 40 to 80 %. Low-boiling solvents are used to reduce the energy requirement for their recovery by distillation and high-boiling solvents are useful for high-temperature treatment but are difficult to recover (Mesa et al., 2016). Dissolving lignin and hemicellulose by Organosolv pretreatment leaves cellulose as crystalline solid. Then, hemicellulose fractions are separate from the solvent as aqueous solution and lignin as precipitate (Shrotri et al., 2017).

Lignocellulose is efficiently fractionated using Organosolv method without significant loss of components or monomeric sugars due to degradation. Therefore, the method is useful for catalytic conversion of lignocellulose as all three components can be processed separately after pretreatment. A drawback of the process is a need of large excess of organic solvent to obtain pure fractions. The recycling of solvents increases the energy demand of the process (Shrotri et al., 2017).

5.4. The Viscose process

Viscose process is a two steps process to obtain regenerated fibres of cellulose. In the first step, cellulose react with the highly toxic carbon disulphide (CS_2). As a result, cellulose xanthate is produced (known as Viscose, containing > 90% of cellulose). Then, in the second step, the xanthate is dissolved in an aqueous solution of sodium hydroxide and cellulose is recovered with sulphuric acid. In this last step, CS_2, sulphur and hydrogen sulphide (H_2S) are liberated. The main advantage of the process is the ability to dissolve high quantities of cellulose. However, only 70–75% of the hazardous chemicals needed for the dissolution can be recovered and re-utilised (Zhang et al., 2018).

5.5. The Lyocell process

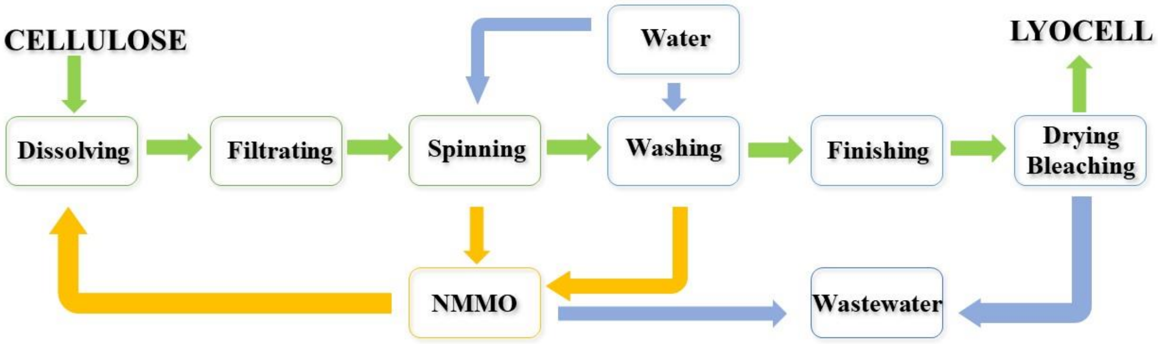


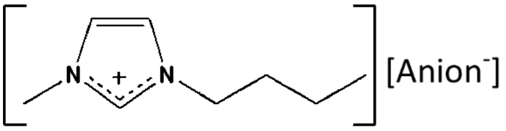
Figure 3. Manufacture process of lyocell fibres adapted from Zhang et al., 2018.

The Lyocell process consists of five steps (Figure 3). During dissolution, cellulose is dissolved in an aqueous system containing N-methyl morpholine-N-oxide (NMMO) to form a solution of a high viscosity. The pulp dissolution in this process is much simpler than that of the Viscose process. Secondly, the solution is filtered to remove coarse components. Thirdly, the solution is extruded through an orifice spinneret into an air gap, and then regenerated in a coagulation bath. Then, the resulting fibres are washed and the residual NMMO is recovered and recycled. Finally, post-treatment fibres are bleaching and drying. The formed fibres are known for their excellent mechanical properties. The major advantage of the process is that NMMO is biodegradable and can be washed out with water and recovered over 99%. The higher disadvantage is that during the process a runaway can be produced during formation of by-products as N-methylmorpholine (NMM) and morpholine (Zhang et al., 2018).

6. Ionic liquids: an alternative to dissolve cellulose

The conventional methods used to recover cellulose from lignocellulosic biomass involve high temperatures and pH, release toxic by-products and provoke large amount of water contamination.

Given this situation, since 2002 ionic liquids (ILs) have been studied and used as an alternative to dissolve cellulose by conventional processes (Swatloski et al., 2002). The ability to work at atmospheric pressure and dissolve any component with well-tuned solvent has spurred significant research into ILs for lignocellulosic processing (Kim et al., 2011). ILs, due to their non-volatile character, are green replacement for harmful volatile organic solvents, they have excellent chemical and thermal stability, potential recoverability and also design possibilities (Plechkova and Seddon, 2008). They are salts composed of large organic cation and an organic or inorganic anion (Figure 4). Finally, at temperatures below 100 ˚C there are in a liquid state (Wahlstrom and Suurnakki, 2015).



[Anion⁻] = [CH_3-O-SO_3^-] [Cl^-]

Figure 4. Example of ionic liquid (1-butyl-3-methylimidazolium methyl sulfate or chloride).

ILs are solvents able to disrupt and break the intermolecular hydrogen bond of the cellulose network allowing its dissolution (Uto et al., 2018). Afterward, the addition of water or other protic solvent including ethanol, methanol, acetone or acetonitrile, causes the instantaneous precipitation of the dissolved cellulose (Wang et al., 2012). This procedure of dissolution of cellulose was widely used from 2002 until now (Swatloski et al., 2002; Pinkert et al., 2009; Gupta and Jiang, 2015; Grøssereid et al., 2019).

Ionic liquids are called “green chemicals (solvents)” due to their possibility to be recovered (Anastas and Eghbali, 2010). The recovery can be done by the evaporation or by the distillation of the solvent used for the precipitation of the cellulose. However, those operations require a large energy input, increasing considerably the cost of reaction. For this reason, it is necessary to design new ionic liquids able to dissolve cellulose and be able to be recovered with minimal energy consuming operations.

7. The bleaching of cellulose

Cellulosic precipitate from waste biomass after extraction with ionic liquids contains ashes, proteins and carbohydrates (cellulose, hemicellulose and lignin). The removal of the proteins and ashes from the carbohydrates contained in the precipitate requires a multi sequential separation.

In the industry, the operation of cleaning of the cellulose is called bleaching. There are two types of bleaching: mechanical and chemical. The main goal of the mechanical bleaching is to make the fibres brighter by eliminating the ashes. The substances commonly used for mechanical bleaching are sodium dithionate and hydrogen peroxide. This operation is able to achieve high brightness (Bajpai, 2018). The aim of the chemical bleaching is to remove the residual lignin. This chemical bleaching is especially applied in the Kraft process (Hintz and Lawal, 2018).

To perform the bleaching of the cellulose recovered from waste biomass, it was necessary to design an operation based on the work of Ahuja (Ahuja et al.2018). The procedure has two steps: treatment with hydrogen peroxide and then with sulfuric acid. Freshly prepared solution of H_2O_2 caused the dissolution of proteins from the recovered cellulose. The hydrolysis with H_2SO_4 resulted in the isolation of the pure cellulose fibers by breaking traces of hemicellulose and lignin into simple sugars (Ahuja et al.2018). The recovered and bleached cellulose was then valorized to a value-added chemical: levulinic acid.

8. Valorisation of cellulose from waste biomass to levulinic acid

8.1. Introduction

In the context of circular economy, the sewage sludge is identifying not as a waste but as a feedstock of valuables components such as lipids, proteins, carbohydrates and metals (Ghergher et al., 2019). Carbohydrates, as cellulose or hemicellulose, are carbon based structural framework renewable feedstock which can be valorized to generate higher value-added chemicals (Badgujar et al., 2019). According to The Pacific Northwest National Laboratory (PNNL), National Renewable Energy Laboratory (NREL) and Office of Energy Efficiency and Renewable Energy (EERE) there are 12 building block molecules that can be obtained from the carbohydrates by bio- or chemo- conversion processes (Werpy and Petersen, 2004). Then, these 12 building blocks (Table 2), also called chemical platforms, are used to generate high value-added chemicals (Badgujar et al., 2019).

Table 2. The twelve sugar-based building blocks.

Nº	**Sugar-based building block**
1.	1,4-Diacids (succinic, fumaric and malic)
2.	2,5-Furan dicarboxylic acid
3.	3-Hydroxy propionic acid
4.	Aspartic acid
5.	Glucaric acid
6.	Glutamic acid
7.	Itaconic acid
8.	Levulinic acid
9.	3-Hydroxybutyrolactone
10.	Glycerol
11.	Sorbitol
12.	Xylitol/Arabinitol

8.2. The levulinic acid

The levulinic acid (LA) is one of the 12 building block chemicals which has gained a lot of interest in the last years. LA contains bifunctional acidic carboxyl and ketonic-carboxyl groups and it is widely used as a precursor in different industries (Morone et al., 2015) (Figure 5).

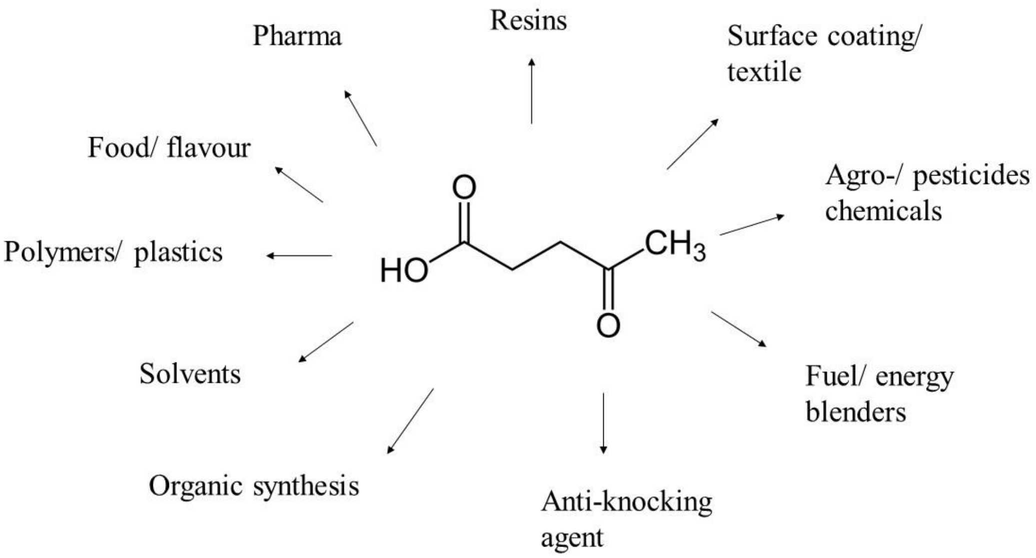


Figure 5. Levulinic acid and its application as a synthetic precursor, adapted from Badgujar et al., 2019.

Levulinic acid can be obtained from biomass waste by cellulose hydrolysis with strong mineral acids. However, due to environmental concern, the strong mineral acids are trying to be replaced by the acidic ionic liquids. The use of ionic liquids let to carry out a one-pot synthesis of LA. The main advantages of using one-pot synthesis are: process efficiency, cost reduction, decrease reaction time and the amount of solvent, furthermore reduction of solvent distillation (Badgujar et al., 2019). The synthesis of LA (Figure 6) starts by depolymerization of cellulose into glucose and hemicellulose into xylose. Glucose isomerizes into fructose. Further dehydration of fructose and xylose let to obtain 5-hydroxymethylfurfural (5-HMF) and furfural, respectively. Then, 5-HMF is rehydrated to LA with formic acid and humins as principal by-products (Badgujar et al., 2019; Velaga et al., 2019).

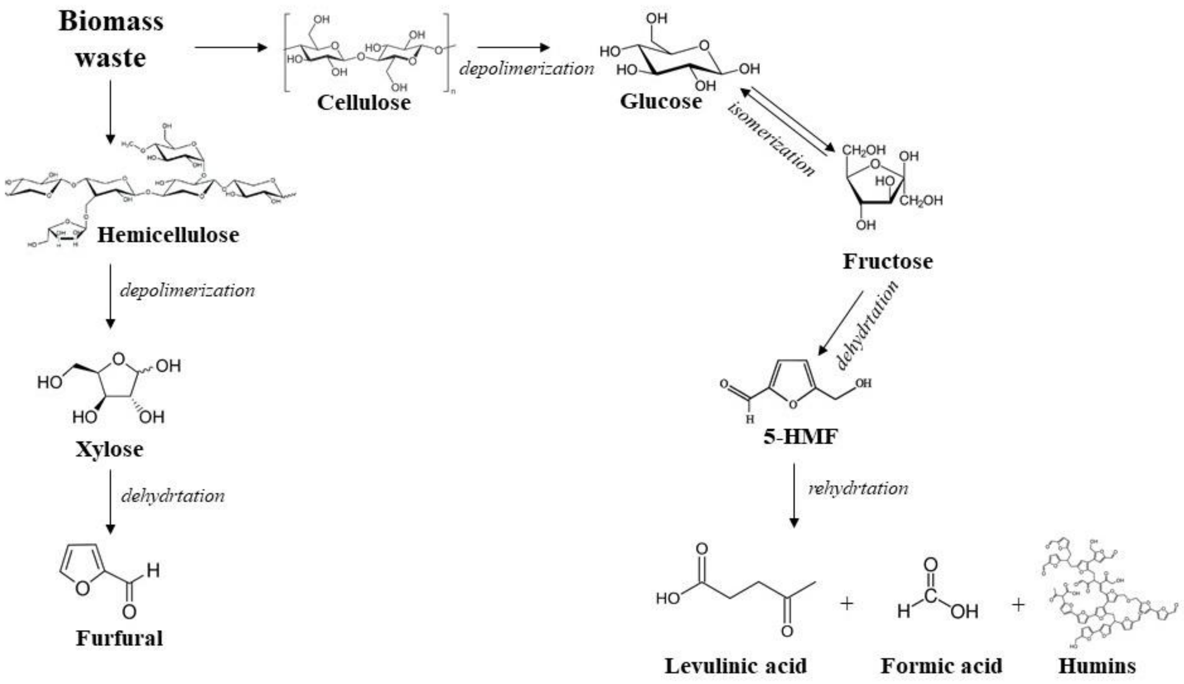


Figure 6. Reaction cascade for the formation of levulinic acid from lignocellulosic biomass waste.

8.3. By-products

The by-products of LA synthesis can be further used in many applications. Formic acid finds utilization in different fields: hydrogen carrier for fuel cells, textile dyeing, food additives, natural rubber, etc. (Kawanami et al., 2017). In turn humins, which are macromolecular insoluble components remained after the reaction, they can be used as composite materials, soil amendment, source of sustainable H_2 and as a model feedstock for gasification (Rasmussen et al., 2014; Kang et al., 2018; Montané et al., 2019).

9. Objectives of the thesis

The overall objective of the fully experimental research work (Figure 7) is the valorisation of waste biomass: municipal sewage sludge, industrial paper sludge and agricultural corn stover. In order to achieve the aim, the following purposes have been done:

- ❖ The investigation of influence of commercial and cheap phosphonium-based ionic liquid on cellulose separation from industrial paper mill wastewater dried sludge (Chapter 2).

- Characterization of paper mill sludge by standard methods; cellulose recovery from paper mill sludge by phosphonium and imidazolium based ionic liquids, and characterization of recovered cellulose (Chapter 3).
- Optimization of conditions used to recover cellulose from primary sewage sludge by phosphonium based ionic liquid (Chapter 4).
- Evaluation of novel synthesised ionic liquid (tetraalkylphosphonium and imidazolium ILs) on cellulose recovery from lignocellulosic material, i.e. corn stover (Chapter 5).
- Application of cellulose recovered from both, urban and industrial sludge to receive value-added products. Reaction catalysed by Brønsted acidic ionic liquid (Chapter 6).

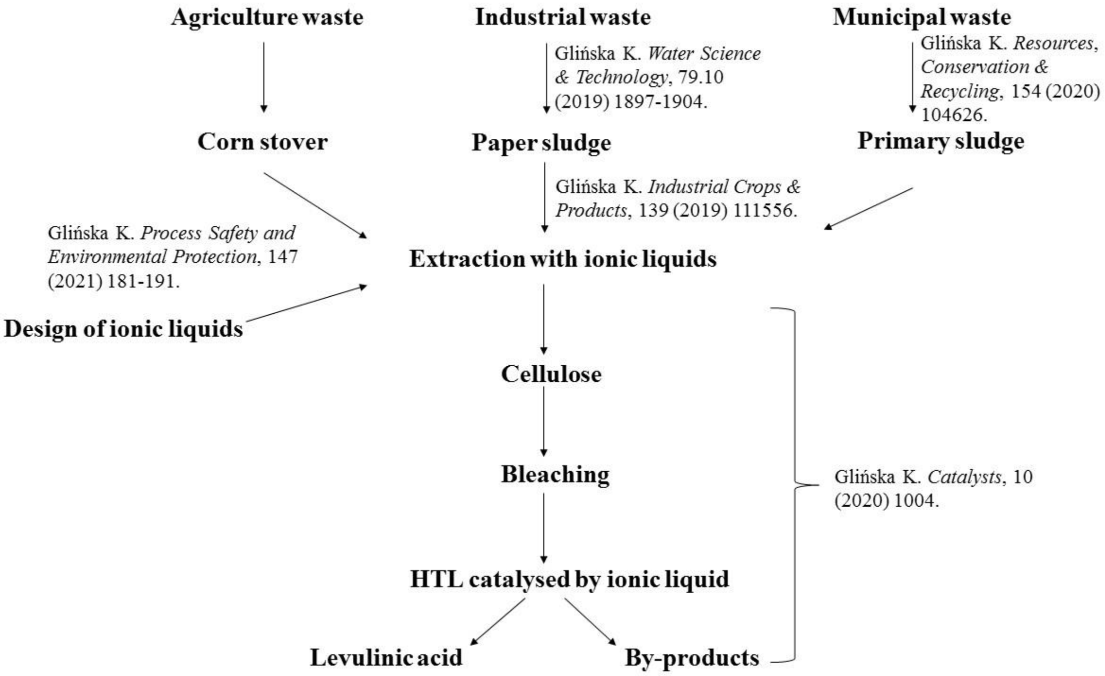


Figure 7. Scheme of fully experimental research work.

10. Thesis outline

The document is organized into seven chapters outlined below:

- Chapter 1 presents the general introduction and the objectives of this work.
- Chapter 2 investigates cellulose separation from industrial paper mill wastewater dried sludge using a commercial and cheap ionic liquid. This work has been published in the Water Science & Technology.

- Chapter 3 describes in detailly the method of cellulose recovery from paper mill sludge by phosphonium and imidazolium based ionic liquids, and also presents full characterization of recovered cellulose. This work has been published in the Industrial Crops & Products.
- Chapter 4 reports an experimental design in order to optimise cellulose recovery from primary sewage sludge with ionic liquid. This work has been published in the Resources, Conservation & Recycling.
- Chapter 5 presents the work done during the research stay at the QUILL group in Belfast, when the feasibility of the synthesised ionic liquids to recover cellulose from lignocellulosic material, i.e. corn stover was studied. This work has been published in the Process Safety and Environmental Protection.
- Chapter 6 investigates the application of recovered cellulose toward into the conversion to value-added chemical (levulinic acid) catalysed by Brønsted acidic ionic liquid. This work has been published in the Catalysts.
- Chapter 7 presents the main conclusions of the thesis and the suggestions for the future work in that field.

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Chapter 2

Separation of cellulose from industrial paper mill wastewater dried sludge using a commercial and cheap ionic liquid [1]

ABSTRACT

Industrial wastewaters and their treatment are now placed at the heart of the environmental concerns that industries face. Some research work has been carried out in order to limit the impact of these wastes on the environment as well as their costs. In this study, wastewater dehydrated sludge (55% wt. water content) from paper industry was used to recover cellulose by using (hydroxymethyl) phosphonium chloride $[P(CH_2OH)_4]Cl$ ionic liquid as a solvent. The ionic liquid has shown remarkable results in terms of cellulose extraction in addition to its non-volatility and lower toxicity compared to organic volatile solvents. All cellulose, based on dry sludge, was recovered from the industrial dehydrated sludge with better operation conditions. The influence of temperature and the quantity of ionic liquid was preliminary studied in order to optimize the extraction conditions.

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[1] Glińska K., Aqlan M., Giralt J., Torrens E., Fortuny A., Montané D., Stüber F., Fabregat A., Font J., Olkiewicz M., Bengoa C. Separation of cellulose from industrial paper mill wastewater dried sludge using a commercial and cheap ionic liquid. *Water Science & Technology* 79.10 (2019) 1897-1904.

1. Introduction

The global production of paper was 400 million tons in 2015. Production is expected to increase to attain a world production of 550 million tons in 2050 (Faubert et al., 2016). The pulp and paper industry is a regular and intensive consumer of water. It is considered as the third water user for its processes, behind the metallurgical or chemical industry (Buruberri et al., 2015). For all this, the paper industry generates large amounts of wastewater in its processes, between 1.5 and 60.0 m^3 per day and ton of paper produced. The treatment of this wastewater also generates between 40 and 50 kg of dried sludge per ton of paper produced (Adhikari and Bhattacharyya, 2015). Of this amount, 70% comes from primary sludge and 30% from biological sludge (Jaria et al., 2017). The management of waste sludge is a serious problem for the paper industry and the solution adopted is usually the incineration of these, often with a high economic cost and the consequent emissions of greenhouse gases (Faubert et al., 2016). Other alternative possibilities widely used are: application in agriculture as fertilizer, building materials or production of bioethanol (Farghaly et al., 2017).

However, the European paper industries, associated with the Confederation of European Paper Industries (CEPI), fully support the European framework directive on waste (2008/98/EC) which establishes a series of recommendations for proper waste management. Among them are the prioritization of recycling over the incineration or deposition in agricultural fields and finally, over the production of energy. Moreover, the European Commission Roadmap to Resource Efficient Europe (COM (2011) 571) foresees the sustainability of Europe's economy by 2050, where waste is managed as a resource and energy recovery can only be used with non-recyclable materials. This last milestone needs to be achieved in 2020 (Molina-Sánchez et al., 2018).

The application of these directives has led to the production of new materials with the sludge produced during the wastewater treatment of the paper industry. Some examples are the production of pesticides and fertilizers or thermal insulation materials (Jaria et al., 2017). Another possibility to enhance the residual sludge is to extract the cellulose, which can then be reused or converted into other materials with higher added value.

There are some conventional processes to recover the cellulose in the pulp and paper industries: Kraft process (Reeve, 2002); Organosolv process (Chundawat et al., 2010); Viscose process (Zhang et al., 2018); Lyocell (NMMO) process (Zhang et al., 2018). All the processes work well but they have some inconveniences, they are harmful to health by the toxicity of the solvents, have a nauseating smell and are pollutant. In addition, products or by-products cannot be easily used and valorised or some of the organic solvents are difficult to recover and reuse. For this reason, it is necessary to find alternatives or new friendlier processes.

Moreover, it is widely known that ionic liquids have great ability to dissolve cellulose and other lignocellulosic biopolymers (Wang et al., 2012). Ionic liquids were successfully used to precipitate cellulose from municipal sewage sludge (Olkiewicz et al., 2015a). Thus, the technical feasibility of using ionic liquids to recover cellulose from an industrial sludge was investigated in the present study.

2. Materials and methods

2.1. Materials

The dehydrated sludge (55% wt. water content) was provided by the paper international company Gomà-Camps, S.A., La Riba, Tarragona, Spain. The company, founded 250 years ago, has two paper fibre production lines, the first one with virgin cellulose pulp and the second with recycled paper. The sludge is a mixture of primary sludge coming from the physic-chemical treatment used to clean the recovered cellulose and the secondary sludge from a membrane biological reactor. Sludge is dried with air, stocked and is used to produce energy.

After reception, the dried sludge was stored at 4ºC in a fridge prior to use. Sludge was used as received. To realise the precipitation of cellulose, the sludge was suspended with deionised water until a final concentration of 5 $g_{\text{dried sludge}}$/L.

The ionic liquid used to recover cellulose from industrial dried sludge was Tetrakis (hydroxymethyl) phosphonium chloride ($[P(CH_2OH)_4]Cl$, hydrated ionic liquid, 80% in water, density 1.34 g/cm^3). It was supplied by Sigma-Aldrich.

Methanol, highest purity sulfuric acid, sodium bicarbonate, n-hexane, hydrochloric acid and magnesium sulfate monohydrate were provided by Sigma-Aldrich. Sodium tartrate, sodium chloride and diluted Folin reagent used for the sludge analyses were also provided by Sigma-Aldrich. The 80% phenol dissolution was prepared fresh. Deionized water was used in all laboratory procedures.

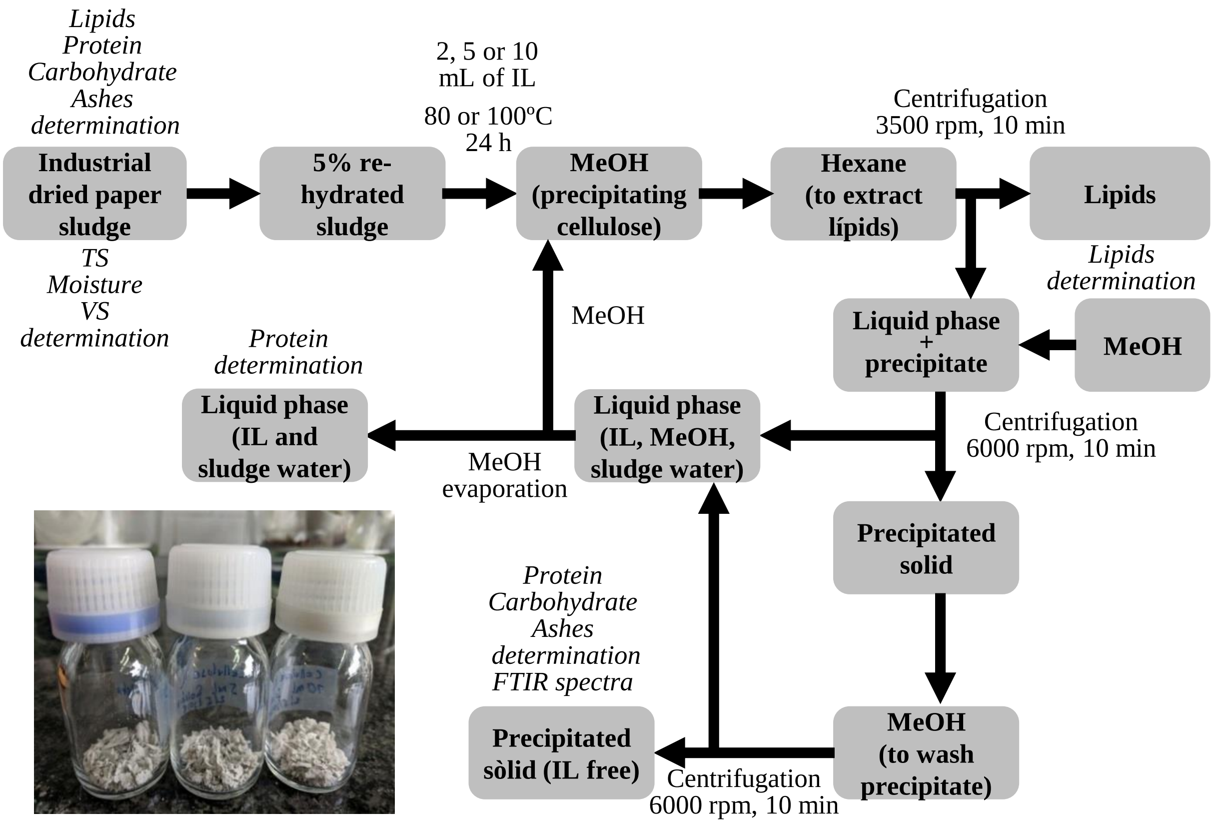

Figure 1. Scheme of the experimental procedure of extraction of cellulose from industrial paper sludge with ionic liquid.

2.2. Experimental procedure

The process of the separation of cellulose from rehydrated dried sludge is summarized in Figure 1. The process was carried out using a similar method conducted for the extraction of lipids from primary sewage sludge (Olkiewicz et al., 2015a). The method is as follow: the necessary volume of rehydrated sludge to have 1 g of total solids was placed in a round bottomed flask. Then, it was added a certain amount of ionic liquid, 2, 5 or 10 mL, depending on the ratio sludge:IL investigated. The flask containing the reagents, wastewater and ionic liquid, was heated at a determined temperature in an oil bath (80ºC or 100ºC) for a fixed duration of time (24 h). The solution was mixed by a magnetic stirring of 500 rpm. The flask was connected with a condenser on the top to condensate the liquid in the process. A blank experiment was realised to see the effect of the ionic liquid on the recovery of carbohydrates. The conditions were: 24h of time, 80ºC of temperature and 1:10 ratio between sludge and water instead of ionic liquid. The rest of procedures were the same.

During the reaction, the sludge was dissolved by the ionic liquid. This dissolution caused the formation of a gel because of the higher viscosity of the solution. When the reaction was over, the mixture was cooled to room temperature. Then, 5 mL of methanol where added to provoke the separation of phases and the precipitation of the cellulose. At that point, 10 mL of hexane were added to the mixture to easily separate the lipids from the rest of phases. The mixture was stirred and placed in a laboratory centrifuge at 3500 rpm for 10 minutes. Two liquid phases were obtained. The yellow organic upper phase contained lipids and hexane. The aqueous liquid phase consisted of methanol, ionic liquid, and water. The precipitated phase containing cellulose was found in the bottom.

Firstly, lipids in hexane were separated using a pipette. Hexane was added again in order to complete lipid separation. The operation was repeated 3 or 4 times until the organic phase became uncoloured after 3 or 4 additions of hexane, which meant that all the lipids had been extracted.

Then, to recover the cellulose, the mixture of aqueous phase and precipitate was homogenized and placed in a laboratory centrifuge at 6000 rpm for 10 minutes. The aqueous phase was eliminated by a pipette. After this, methanol was added to the aqueous phase to eliminate the ionic liquid in the precipitated phase and centrifuged again. The process was repeated 3 or 4 times until the ionic liquid was totally eliminated in the aqueous phase which was signed by a white colour. The aqueous phase recovered from the extraction was also filtered to separate the traces of the precipitated phase. The precipitated solid obtained after filtration was put in an oven at 105ºC of temperature for 24 hours in order to measure the total solid.

The final solid obtained after elimination of the aqueous and the organic phase contained traces of methanol. The methanol in the solid phase was evaporated in a rotary evaporator. The solid obtained was kept in a desiccator to avoid absorption of water. The solid phase or precipitate was then weighed.

To see the influence of the sequence on the operative protocol, another experiment was realised, modifying the protocol of separation, centrifuging just after heating. The rest of the procedures were exactly the same, just adding methanol, then hexane, to both phases, solid phase and supernatant.

Total solids (TS), volatile solids (VS) and ash content were analysed to characterize the precipitate following the procedures described in the analytical characterization. A sample of precipitated solid was then examined by Fourier Transform Infrared (FTIR) spectroscopy. The yield of precipitate was calculated based on 100 g total solid equivalent as:

$$\text{Precipitate yield \%} = \frac{\text{Precipitate obtained [g]} \times \text{its fraction of dry weight}}{\text{wet sludge [g]} \times \text{its fraction of dry weight}} \times 100$$

2.3. Analytical characterization

Total solids (TS) and volatile solids (VS) were both determined by the standard method 2540B (Rice et al., 2012). Ash content was also defined as described by the standard method 2540E (Rice et al., 2012).

The total carbohydrate composition was determined using phenol-sulfuric acid according to Dubois method (Dubois et al., 1956), the absorbance was read at 480 nm.

Protein composition was determined by the Lowry method (Lowry et al., 1951), the absorbance was read at 750 nm.

Lipid composition from dried sludge was evaluated using a classical methodology with a Soxhlet apparatus and hexane as a solvent according to standard method 5520E (Rice et al, 2012). Around 20 ml of wet sludge was first acidified to pH=2, then 25 g of dry $MgSO_4,H_2O$ were added in order to eliminate the water and obtain an homogenous paste. The sludge was then left for 30 minutes. The solid obtained was crushed into fine powder. The powder was placed in a cellulose extraction thimble and coved with a glass wool. The extraction was then conducted using a Soxhlet and hexane solvent at 20 cycles/hour during 4 hours. Afterwards, the hexane was evaporated using a rotary evaporator. The lipids were stored in a desiccator to avoid water absorption and weighed on the next day. The lipid yield was expressed as gram of lipids per gram of dry sludge. The following equation shows how lipid yield was calculated:

$$\text{lipid yield \%} = \frac{\text{fats and oils [g]}}{\text{wet sludge [g]} \times \text{fraction of dry solid}} \times 100$$

The precipitated solid was analysed by Fourier Transform Infrared (FTIR) spectroscopy to check the presence of carbohydrates. 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

Table 1 presents the results obtained for the characterisation of the dried industrial sludge. As it can be seen in the table, sludge was not totally dried as it contains 56% of water.

Table 1. Characterisation of industrial paper wastewater dried sludge.

	Characterisation of dried sludge [a] (%)	**Mass of the fractions of dried sludge (g) [b]**
Moisture	56.0 [c]	56.0
Total solids (TS)	44.0 [c]	44.0
Ashes	45.4 ± 1.1 [d]	20.0
Volatile solids (VS)	54.6 [d]	24.0
Carbohydrates	54.8 ± 3.0 [d]	24.1
Protein	2.6 ± 0.2 [d]	1.1
Lipids	1.0 ± 0.2 [d]	0.4
Total	103.8 [d,e]	45.6 [e]

Values are mean, ±SD, n=3

[a] All analysis according to conventional methods; ashes-2540B (Rice el al., 2012), proteins – Lowry method, carbohydrates - Dubois method, lipids by extraction with Soxhlet apparatus.

[b] Calculated on basis of 100 g of sludge as received.

[c] (%, w/w_{sample}).

[d] (%, w/w_{TS}).

[e] Sum of ashes, carbohydrates, protein and lipids (corresponding to total solids).

Total solids represent 44% of the sludge. The composition of the total solids is 54.6% (w/w_{TS}) of volatile solids and 45.4% (w/w_{TS}) of ashes. There is high value of ashes because the sludge contains inks and other metallic substances extracted during the washing of the recovered paper. The composition of volatile solids is essentially carbohydrates, 54.8% (w/w_{TS}), basically cellulose, proteins, 2.6% (w/w_{TS}) and lipids, 1.0% (w/w_{TS}). The values of proteins and lipids is essentially due to the presence of micro-organisms from the secondary membrane bioreactor used to treat wastewater. This composition is totally different of the composition of primary sludge (Olkiewicz et al., 2015a) where lipids accounted for 27.2% (w/w_{TS}), proteins for 24.2%

(w/w_{TS}) and carbohydrates for 26.2% (w/w_{TS}). As expected, the composition of carbohydrates (cellulose) is higher in the industrial sludge but the high presence of ashes implies a better cleaning of the precipitate to allow the possibility to reuse cellulose.

In Table 2, the yields of cellulose and ash in precipitated material obtained after extraction of raw primary sludge with the ionic liquid are presented. The values are expressed as a percentage based on dry paper sludge. The results are obtained after reaction with 2 different temperatures using the same amount of ionic liquid (1:10 mL) and the same time of reaction (24h). In the first case (80ºC) it was obtained 52.6% (w/w_{TS}) of cellulose and 10.6% (w/w_{TS}) of ash whereas at 100ºC it was obtained 53% (w/w_{TS}) of cellulose and 11.1% (w/w_{TS}) of ash. As it can be seen in Table 2, there is no influence of temperature on the cellulose and ash yield in the precipitated material, in both cases similar results were obtained, however lower temperature conditions may have some effect and will be studied in the future.

Table 2. Influence of extraction temperature on the yield of cellulose from industrial paper sludge using [P(CH_2OH)$_4$]Cl ionic liquid (24 h, 1:10 ratio sludge:IL).

			Precipitated material (%, dried sludge)	
Temperature (ºC)	**Time (h)**	**Ratio sludge:IL (g_{TS}:mL_{IL})**	**Cellulose**	**Ashes**
80 B.E.	24	1:10 (water)	48.8	37.0
80	24	1:10	52.6	10.6
80 P.M.	24	1:10	44.3	7.2
100	24	1:10	53.0	11.1

B.E.: Blank experiment; P.M.: Protocol modified, centrifugation just after 24 h heating.

The results of the blank experiment to see the effect of the ionic liquid on the recovery of carbohydrates are also presented in Table 2. The unique difference was the use of water instead of ionic liquid in the 1:10 ratio experiment. As it can be seen in the table, the recovery of carbohydrates is slightly lower but the quantity of ashes is more or less quadrupled. This demonstrates that the effect of ionic liquid is especially important on the separation of ashes from carbohydrates. The design of new ionic liquids must increase this separation until there is no presence of ashes in the carbohydrates.

The results of the experiment with a modified protocol are also presented in Table 2. The unique modification was to centrifuge the mixture just after heating. After centrifugation, methanol was added to both phases solid and liquid. In the liquid phase containing the ionic liquid nothing occurred. No additional solid precipitated, this signifying that all carbohydrates were in the solid phase. The ashes in this liquid phase, calculated by difference, where 38.4% dried sludge basis. This value is similar to the classical protocol. The addition of methanol in the solid phase, and after mixing, did not allow the separation of carbohydrates from the rest of ashes. The content of ashes in the solid phase was 7.2% dried sludge basis, a little less than the obtained in the other experiments with classical protocol. Moreover, the addition of hexane to liquid and solid phases to recover lipids, showed that all lipids were in the solid phase. The lipids where recovered as in the classical protocol, the obtained value was similar, 0.8% dried sludge basis. This demonstrates that the sequence of operation does not have a great influence in the results of the separation. Prior centrifugation does not really improve the complete separation of compounds. Again, right design of ionic liquid will be required for a total separation.

In Table 3 we see the results obtained after reaction with 3 different ratios of sludge:IL (2, 5 and 10 mL) using the same temperature (100ºC) and the same time of reaction (24h). As it is shown in Table 3, the cellulose and ash content depends on the amount of ionic liquid used. The less volume of ionic liquid was used, the more cellulosic material was obtained. With 2 mL of ionic liquid it was obtained 56.9% (w/w_{TS}) of cellulose and with 10 mL, 53.0% (w/w_{TS}) of cellulose. However, lower amount of ionic liquid (1:2 sludge:IL ratio) gave higher amount of ashes, 18.3% (w/w_{TS}), in the precipitated material, resulting on a cellulose material more contaminated with ashes. According to the results, the optimum ratio of sludge to ionic liquid is 1:5 (g_{TS}:mL_{IL}) giving high amount of cellulose and low amount of ashes.

Table 3. Influence of ratio sludge:IL on the yield of cellulose from paper sludge using $[P(CH_2OH)_4]Cl$ ionic liquid (100ºC, 24 h).

			Precipitated material (%, dried sludge)	
Temperature (ºC)	**Time (h)**	**Ratio sludge:IL (g_{TS}:mL_{IL})**	**Cellulose**	**Ashes**
100	24	1:2	56.9	18.3
100	24	1:5	56.1	9.0
100	24	1:10	53.0	11.1

Table 4 shows the full characterisation of the entire process of recovery of cellulose by the ionic liquid. The experimental conditions were: 100ºC of temperature, 24 hours of reaction and 1 g_{TS}:5 mL_{IL} sludge:IL ratio. The first phase to be quantified were the lipids. After separation of organic upper phase, hexane was evaporated and lipids were weighted. The obtained value, 1.1% w/w_{TS}, is almost identic to the value of lipids obtained by soxhlet extraction and presented in Table 1, 1.0% w/w_{TS}. The separation of lipids obtained with ionic liquid was complete, signifying that ionic lipids are more or less able to separate all fractions from the sludge in a single operation.

The determination of protein in the liquid aqueous phase by Lowry method (Lowry et al., 1951) provided a value of 1.4% w/w_{TS}. This value is more than 50% of the protein initially contained in the sludge, proving that the ionic liquid used for the recovery of cellulose is not totally selective for the separation of cellulose. The content of protein in the solid phase was determined by mass balance.

As it can be seen in the table, the ionic liquid was also unable to eliminate all ashes in the precipitate. The amount of ashes found in the precipitate, 9.0% w/w_{TS} is more or less 20% of the total ashes contained in the sludge. The presence of these ashes are responsible for the final slightly dark colour of the precipitate.

The carbohydrates content in the precipitated solid after separation with ionic liquid was calculated from the difference between the value of volatile solids in the precipitate and the proteins in precipitate. The value of carbohydrates obtained by mass balance, 56.1% w/w_{TS} is close to the initial carbohydrate content measured by Dubois method (Dubois et al., 1956) and presented in Table 1, 54.8% w/w_{TS}. Considering the accuracy of these results, it is possible to say that almost all the cellulose is in the precipitate. This is very important because a commercial ionic liquid is able to separate all the cellulose from a wastewater sludge. Specific design of new ionic liquids must be able to decrease the quantities of ashes and protein in the precipitate.

Table 4. Characterisation of the fractions after their separation by ionic liquids (100ºC, 24 h, 1:5 sludge:IL ratio).

	Organic phase	**Liquid aqueous phase containing the ionic liquid**	**Solid phase: precipitate**
Ashes (%, w/w_{TS})	-	36.4	9.0
Carbohydrates (%, w/w_{TS})	-	-	56.1 [a]
Proteins (%, w/w_{TS})	-	1.4	1.2 [b]
Lipids (%, w/w_{TS})	1.1	-	-
Total (%, w/w_{TS})		105.2	

[a] Calculated from the difference between the volatile solids in the precipitate and the proteins in the precipitate.

[b] Calculated from the difference between the total proteins obtained from the dried sludge and the protein analysed in the liquid aqueous phase.

In order to confirm the structure of the obtained solid, cellulose sample and precipitated solid were analysed by Fourier Transform Infrared (FTIR) spectroscopy. Figure 2 presents the spectra of the precipitated solid after extraction of cellulose with ionic liquid. The broad peak around 1035 cm^{-1} (assigned as the C-O stretching vibration of carbohydrates) is presented in both samples. Also, a peak at 1160 cm^{-1} in both spectra, which is assigned to C-O-C stretching vibration is particularly associated with cellulose. Based on FTIR data it could be said that precipitated solid after ionic liquid extraction contains cellulose.

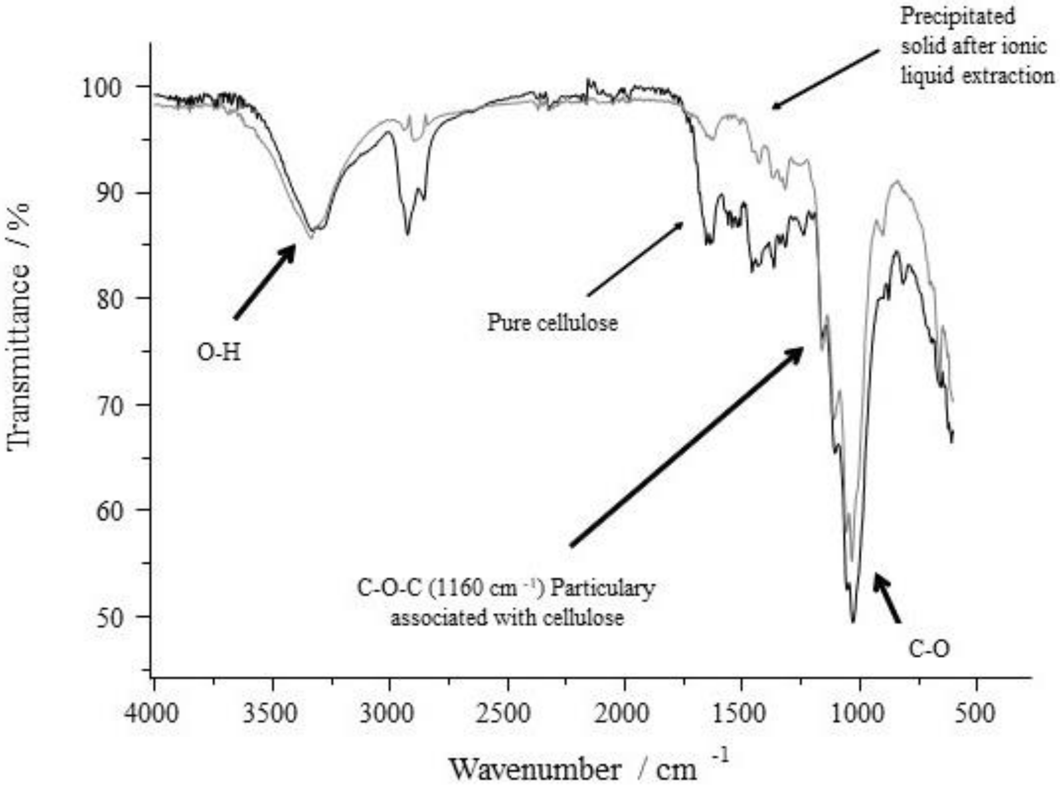

Figure 2. FTIR analysis of precipitated solid after ionic liquid extraction and of pure cellulose.

Additionally, the possibility of recoverability and reusability of the ionic liquid in a new cycle of purification is very important because the energetic and economic balance of the process. In the current work, recoverability and reusability of the ionic liquid were not checked as this study is a fast-technical feasibility one. This was investigated in an older work using the same ionic liquid to extract lipids from microalgae (Olkiewicz et al., 2015b). In this work, the stability of the ionic liquid was tested by ^{1}H NMR spectroscopy, comparing fresh and used ionic liquid. The same peaks were found signifying a high steadiness of the ionic liquid. No supplementary peaks appeared for the reused ionic liquid, suggesting its stability and allowing reusability. The recycling and reuse of the recovered ionic liquid was examined by carrying out four runs. All tests showed similar high performances of the recovered ionic liquid than fresh ionic liquid, allowing its suitability in repeated processes.

Concerning the feasibility and economy of the process, the cost of ionic liquid, temperature and time of reaction of process are parameters that make the practical application of the process questionable when compared to currently applied processes. It is true that the cost of the ionic liquid is not cheap. But this work is a proof of concept. It is a first evaluation of the possibility to recover cellulose from industrial paper wastewater or sludge. The process is not yet optimised and needs a lot of extra work. For example, more selective and green ionic liquids should be optimised and designed, temperature should be decreased, time of reaction should be shortened, ratios sludge:IL should be improved and surely other parameters. Furthermore, this work was performed with dried sludge. As it was demonstrated with municipal primary sludge in previous works, it is possible to work directly with wet sludge, avoiding high costs of drying operation (Olkiewicz et al., 2015a). The only thing that is currently questionable is the full recovery of ionic liquid. Separation and purification of ionic liquid after the recovery of the cellulose is a challenge. However, it has been demonstrated that ionic liquid can be reused as commented in the previous paragraph. Further studies with membrane separation processes might be able to recover the ionic liquid. Then, the problem of cost will also be solved. Finally, it is well known that the scale-up of the process to a semi-industrial continuous setup will improve the performances of the process. When all will be done, it will be then possible to compare the process with other currently applied processes.

4. Conclusions

The ionic liquid tetrakis hydroxymethyl phosphonium chloride $[P(CH_2OH)_4]Cl$ has shown potential for the recovery of the cellulose from the industrial paper sludge. The amount of carbohydrates measured by the conventional method in the paper sludge and the amount of cellulose recovered using ionic liquid method are comparable. The ionic liquid is able to dissolve ashes from paper sludge. The study shows dependence between quantity of ionic liquid used for reaction and amount of cellulose recovered. The less ionic liquid used, the more cellulose obtained. It is necessary to study milder reaction conditions to optimise the process in order to reduce energy and therefore the cost of the process. It is necessary to study a purification process to further clean the cellulose.

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Chapter 3

Recovery and characterisation of cellulose from industrial paper mill sludge using tetrakis and imidazolium based ionic liquids [1]

ABSTRACT

Paper industry is a sector that generates large volumes of wastewater and sludge. There is a possibility to recover the cellulose contained in these effluents. Two tetrakis and imidazolium based commercial ionic liquids were used to recover the cellulose from industrial paper dried sludge. Both ionic liquids were able to recover the cellulose of the sludge (~100%). The ratio sludge:ionic liquid had only a low influence on the recovery. The mass of cellulose recovered was the same for both ionic liquids. The tetrakis ionic liquid was able to clean the precipitate better, eliminating a major quantity of ashes. The imidazolium ionic liquid was able to leave a minor quantity of initial proteins in the precipitate. These encouraging results demonstrate that the recovery of cellulose from paper industry sludge is feasible with ionic liquids. But, improvement of the performances of the ionic liquids is necessary to allow a scale-up of the technology.

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[1] Glińska K., Solehin Bin Ismail M., Goma-Camps J., Valencia P., Stüber F., Giralt J., Fabregat A., Torrens E., Olkiewicz M., Bengoa C. Recover and characterization of cellulose from paper mill sludge using tetrakis and imidazolium based ionic liquids. *Industrial Crops & Products*, 139 (2019) 111556.

1. Introduction

Paper industry is one of the major users of water; in fact, it occupies the third position (Buruberri et al., 2015). It is also one of the industries which apply the concept of circular economy, where material streams maintain added values for as long as possible (Deviatkin et al., 2016). The European pulp and paper industry replaces up to 72% of virgin fibre with recovered paper, normally from municipal and industrial selective garbage collection. Taking into account that 22% of the paper cannot be recovered, the limit will be attained soon (European Recovered Paper Council, 2014). The world production of paper in 2015 was 400 million tons, 83 million in 2017 in Europe. Previsions are about 550 million tons in 2050 (Faubert et al., 2016). The utilisation of recovered paper implies cleaning it. The deinking process generates 150 kg of dry solids by ton of produced paper (Deviatkin et al., 2016). Production of virgin pulp and deinking activities generate large volumes of wastewater, which is called paper mill wastewater. The production of this wastewater is considered to be between 1.5 and 60 m^3 per day per ton of paper produced (Adhikari and Bhattacharyya, 2015). The management of this huge amount of wastewater has a great impact on the environment and requires treatment before its disposal. One of the typical solutions is incineration, which has a high economic cost due to its high-water content and emission of greenhouse gases (Faubert et al., 2016). Other possibilities are disposal in agriculture as fertilizer, production of bioethanol or production of new materials (Farghaly et al., 2017). In fact, this situation is not used anymore and, for this reason, the European Community published the European framework directive on waste (2008/98/EC): “This Directive lays down measures to protect the environment and human health by preventing or reducing the adverse impacts of the generation and management of waste and by reducing overall impacts of resource use and improving the efficiency of such use”. The directive established a hierarchy of actuation, prioritising prevention of waste; re-use of waste; recycling; other recovery, e.g. energy recovery; and finally, disposal. This provoked a change in the usual management of paper mill wastewater. From the beginning, the directive was supported by the Confederation of European Paper Industry (CEPI, www.cepi.org).

On the other hand, the European Commission Roadmap to Resource Efficient Europe (COM (2011) 571) envisages Europe economical sustainability by 2050, by preconizing that waste must be primarily managed as a new resource. Also, utilisation of these wastes as energy vectors is forbidden, allowing it only for non-recyclable materials, and some of these achievements must be completed by 2020 (Molina-Sánchez et al., 2018). This recommendation incited to use paper mill wastewater in the manufacturing of thermal insulation materials, fertilizers or pesticides (Jaria et al., 2017). These processes are not using directly the cellulose contained in the sludge, they are using the sludge. For this reason, processes using the extracted cellulose from sludge, which could be directly re-used for the production of paper or, converted into bio-blocks and new materials will have a higher added value. Thus, the recovery of cellulose from waste paper mill sludge should be prioritised.

There are some conventional processes used for chemical pulping and lignocelluloses wastes that can be used for the recovery of cellulose from the residue produced in the pulp and paper industries. One of those processes is the Kraft process. This process is also known as kraft pulping or sulphate process. It is an industrial process to convert wood into wood pulp consisting of almost pure cellulose fibres. The process treats the wood chips with a liquor, a mixture of sodium hydroxide (NaOH) and sodium sulphide (NaS_2). The liquor breaks the bonds that link lignin to the cellulose allowing its separation. The process has the advantage of being cheap, but the alkaline chemicals used for the process cause metal corrosion and malodorous smells. And more important, the lignin obtained contains sulphur which increases the difficulty for valorisation and requires a higher need for bleaching (Reeve, 2002). Another conventional process is the Organosolv process. It is a pulping technique that uses an organic solvent (acetone, methanol, ethanol, butanol, ethylene glycol, formic acid, and acetic acid) to solubilise lignin and hemicellulose at high temperatures, from 140 to 220°C. The concentration of solvent in water ranges from 40 to 80%. The process has the advantage of producing a sulphur-free lignin, but it has a high cost and it has safety concerns about the utilisation of flammable and toxic organic solvents that can be released to the atmosphere by process emissions (Chundawat et al., 2010). An alternative is the Viscose process. This process is used to manufacture regenerated fibres, obtained by the reaction of cellulose with the high toxic carbon disulphide (CS_2) producing cellulose xanthate (known as Viscose, high cellulose content >90%). The xanthate is dissolved in a sodium hydroxide aqueous solution and cellulose is recovered with sulphuric acid. In this last step, CS_2, sulphur and hydrogen sulphide (H_2S) are liberated. The main advantage of the process is the ability to dissolve high quantities of cellulose but, only 70-75% of the hazardous chemicals needed for the dissolution can be recovered and re-utilised (Zhang et al., 2018). Finally, the Lyocell (NMMO) process is the most recent process used to dissolve the cellulose. Waterfree N-methylmorpholine-*N*-oxide is used as a solvent for the dissolved cellulose that can be recovered by precipitation after addition of water. The formed Lyocell fibres are known for their excellent mechanical properties. The major advantage of the process is that NMMO is biodegradable and can be washed out with water and recovered over

99%. The higher disadvantage is that during the process a runaway can be produced during formation of by-products as N-methylmorpholine (NMM) and morpholine (Zhang et al., 2018). Summarising, the utilisation of classical processes to extract cellulose has more inconveniences than advantages, essentially because of the use of toxic and dangerous organic solvents.

The ionic liquids are the alternative to dissolve cellulose. They are salts composed of a large organic cation and an organic or inorganic anion. Ionic liquids are not volatile, except at very low pressures and high temperatures. They are in liquid phase below 100°C, having, an excellent chemical and thermal stability. Finally, they are not flammable substances (Plechkova and Seddon, 2008). Ionic liquids, compared with conventional treatment methods, have an extraordinary capability to liquefy cellulose and lignocellulosic materials (Kuroda et al., 2014; Loow et al., 2017) without destroying lignin and hemicellulose. Furthermore, cellulose dissolution by ionic liquids does not involve utilisation of high temperatures and pH, as it is in a case of classical processes. The biggest advantage of using ionic liquids is that during the cellulose dissolution there is no release of toxic by-products and water contamination. Recently, ionic liquids were used to precipitate cellulose from urban primary sludge (Olkiewicz et al., 2015a). Thus, it was logic to apply this methodology to other sludge wastes containing high quantities of cellulose. Paper mill wastewater is an ideal candidate to check the methodology. To the best of our knowledge, this is the first time that 1-butyl-3-methylimidazolium methyl sulphate and tetrakis (hydroxymethyl) phosphonium chloride ionic liquids have been used to recover the cellulose from ~~a~~ dehydrated paper industry sludge. To do so, regarding the good results obtained in a previous work with a municipal primary sludge (Olkiewicz et al., 2015a), cheap and commercially available ionic liquids were used to evaluate the recovery of cellulose from ~~a~~ dried paper mill sludge. The effect of the ratio mass of sludge over volume of ionic liquid was also assessed. A full characterisation of the phases after separation was also performed.

2. Materials and methods

2.1. Sludge

The dehydrated sludge (55% wt. water content) was provided by the international paper company Gomà-Camps, S.A., La Riba, Tarragona, Spain. The company was founded 250 years ago. Tissue paper production is a particular papermaking process prone to produce sanitary and hygienic paper such as kitchen and hand towels, napkins or toilet paper. Tissue paper can be produced either with virgin pulp or with recovered fibre sources. The mill that supplied sludge for the study produces paper mainly from recovered fibre. The recycling process consists on separating the contaminants coming from the waste paper. Early separation stages are dedicated to remove dense contaminants through cleaning operations with hydro cyclones, once these contaminants are removed, a screening stage using 0.12 mm slots is necessary to prepare the pulp for its deinking. When the pulp slurry is free from big and heavy contaminants it is then deinked by means of flotation, a classical pure mechanical separation of pulp and ink with air and water. After this, the pulp goes through further hydro cyclonic cleaning stages and a washing step prior to a final contaminant dispersing step to eliminate specks and sticky particles. Once the pulp, the fibre slurry is clean, it is sent to form a wet web of paper that will be pressed and dried prior to reel it up. Water is the conductor of the whole process and it is reused all over it. To do so, water is clarified through dissolved air flotation steps. All the removed contaminants form the screening section and the sludge generated by the water clarifiers are mixed and dehydrated together with the sludge coming from the wastewater treatment plant. The dehydrated sludge is sent to produce compost and the water extracted from it, reused in the mill. As a last stage after the wastewater treatment plant, there is a biological treatment plant which consist mainly of a submerged membrane bioreactor. Water coming off is then partially recycled to the process, as fresh water, and the rest sent to the river. A scheme of the process is presented in Figure 1. After reception, dried sludge was stored at 4ºC prior to use. Sludge was used as received. To realise the precipitation of cellulose, the sludge was dissolved with deionised water until a final concentration of 5 $g_{\text{dried sludge}}$/L.

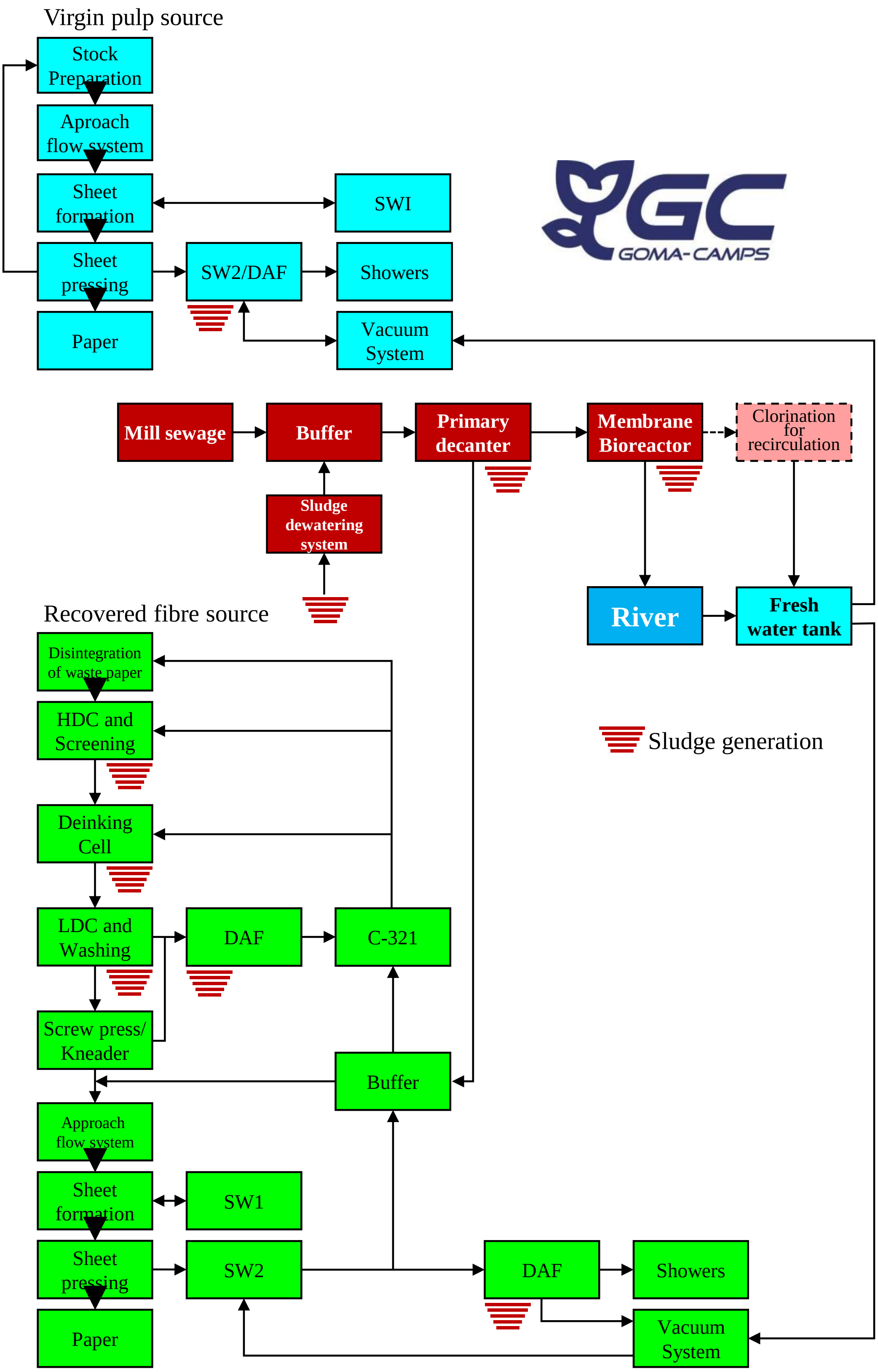


Figure 1. Scheme of the processes of Goma-Camps to obtain paper tissue from virgin pulp and recovered paper.

2.2. Ionic liquids

Ionic liquids used for the recovery of the cellulose from the paper mill sludge were, 1-butyl-3-methylimidazolium methyl sulphate (ref. 53177, >95% purity, 1.21 g/cm^3 density), [C4mim][$MeSO_4$] and tetrakis (hydroxymethyl) phosphonium chloride (hydrated ionic liquid, ref. 404861, 80% in water, 1.34 g/cm^3 density), [$P(CH_2OH)_4$]Cl. Both ionic liquids were supplied by Sigma-Aldrich and used as received. From now on, ionic liquids will be denominated as imidazolium and tetrakis respectively.

2.3. Other materials

Methanol (ref. 32,213), highest purity sulfuric acid (ref. 30743, 95.0-97.0%), sigmacell cellulose (ref. S3504), sodium bicarbonate (ref. 222,321), n-hexane (ref. 15671, high performance chromatography grade), hydrochloric acid (ref. 84,418), fuming (high analytical reagent grade), anhydrous sodium sulfate (ref. 1,614,807) and magnesium sulphate monohydrate (ref. 434,183) were provided by Sigma-Aldrich. Potassium sodium tartrate tetrahydrate (ref: 217,255), sodium hydroxide (ref. S5881) and diluted Folin&Ciocalteu's phenol reagent (ref. F9252) used for the sludge analyse were also provided by Sigma-Aldrich. Phenol dissolution was prepared fresh with phenol crystalline (ref: 144,852.1211) provided by PanReacAppliChem. Deionized water was used in all laboratory procedures.

2.4. Experimental procedure

The procedure of recovery of cellulose from the industrial paper mill sludge was carried out using a comparable method conducted for the extraction of lipids from primary sewage sludge presented in a previous work (Olkiewicz et al., 2015a). The procedure is shown in Figure 2. The recovery of cellulose was performed in a 50 mL round bottomed flask, connected with a condenser on the top to condensate possible vapours formed during process. 1 *g*, exactly, of equivalent total solids (TS) of rehydrated sludge (~20 mL of rehydrated sludge) were placed into the flask, then, 2, 5 or 10 mL of desired ionic liquid were added. The mixture was stirred with a magnetic device at 500 rpm. The flask was heated to 100ºC with an oil bath and the process was realised during 24 h. After this time, the oil bath was removed and the flask cooled down to room temperature. At that point, 10 mL of methanol were added to precipitate the solubilised cellulose. Then, 5 mL of hexane were added to wash the mixture and allow the separation of the lipids. The mixture was divided in several centrifugation tubes and centrifuged during 10 min at 3500 rpm. Three phases were obtained, the upper, a dark yellow or pink organic liquid phase (depending on the ionic liquid), containing the hexane and the lipids. The intermediate, was an aqueous light brown phase containing methanol, ionic liquid, part of the ashes and part of the protein. The last one, the solid brown phase, was the precipitate, containing almost all the cellulose, part of the ashes and part of protein.

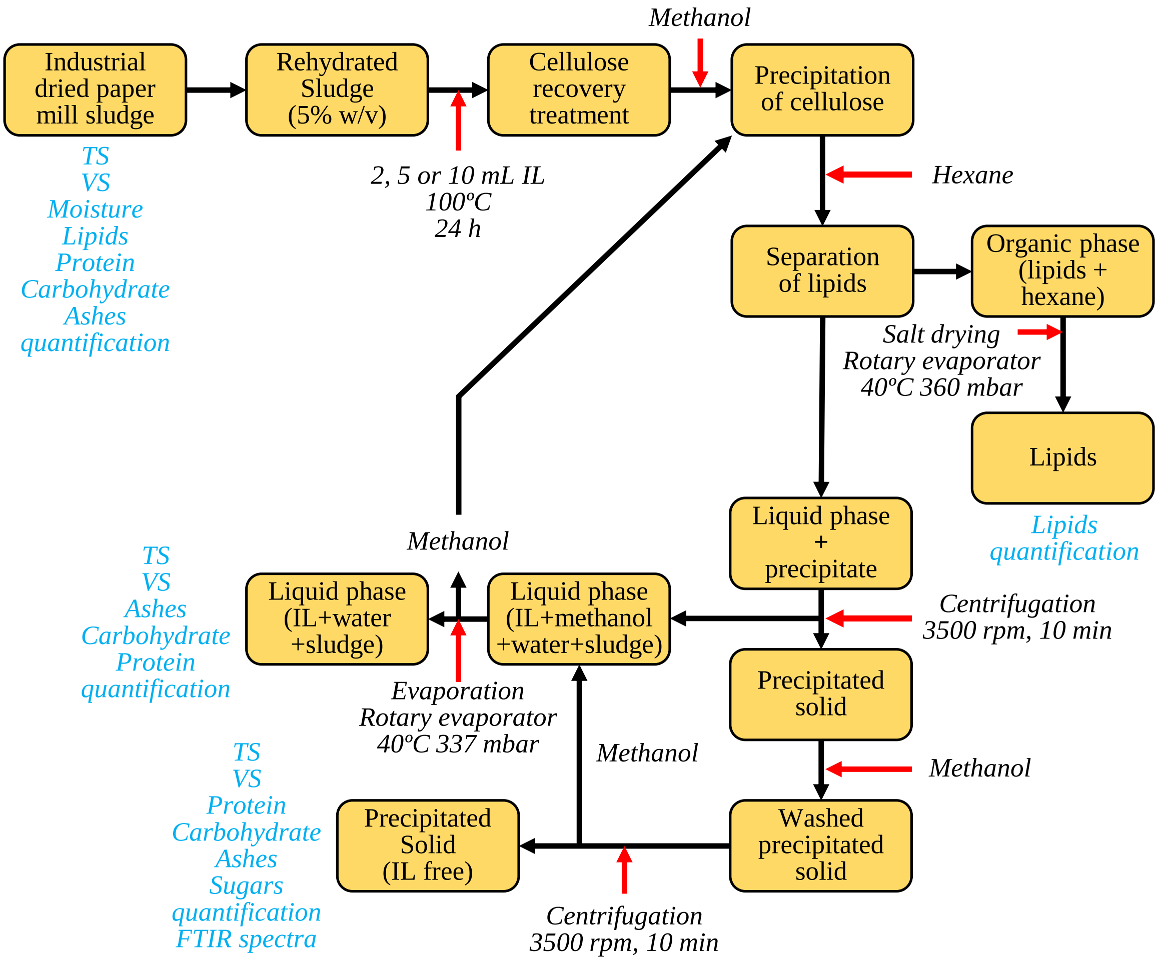


Figure 2. Scheme of the experimental procedure of extraction of cellulose from industrial paper sludge with ionic liquid.

The upper liquid organic phase was removed with a pipette. 5 mL of hexane were used to clean again the mixture, this operation was repeated until the organic phase was transparent signifying that there was no presence of lipids. At this point, the organic washing liquid was added to the lipid phase, then, anhydrous sodium sulphate was added with a spatula to the organic liquid to remove all methanol and ionic liquid. The organic liquid with the salt were maintained during 30 min. The mixture was filtered and the hexane removed with a rotary evaporator working at 40ºC and 360 mbar. The lipids were kept in a desiccator over night to eliminate the last traces of hexane.

The aqueous liquid phase containing the precipitate was washed with methanol to remove the ionic liquid from the precipitate. The mixture was homogenized and centrifuged 10 min at 6000 rpm. The liquid phase was removed with a pipette, the procedure was repeated until the IL was totally eliminated from the precipitate, verified by the transparent colour of liquid. The rest of the methanol remaining in the precipitate was removed with a rotary evaporator working at 40ºC and 337 mbar. The precipitate was kept in a desiccator overnight and weighed the next day.

After the organic liquid phase was removed, the aqueous phase contained methanol, ionic liquid, water, traces of carbohydrates and ashes. Then, the aqueous liquid phase was mixed with 10 mL of methanol. The mixture was stirred and placed in a laboratory centrifuge at 3500 rpm for 10 min. The aqueous liquid phase was fully characterised: total solids, volatile solids, protein, carbohydrates and ashes were determined. The aqueous liquid phase had a particular colour (red for the tetrakis ionic liquid and pink for the MMAM ionic liquid). Then 10 ml of methanol were again added to the aqueous phase in order to eliminate all the ionic liquid, and centrifuged. The ionic liquid was separated with a pipette and the operation was repeated as many times as necessary until the aqueous phase became transparent. During the recovery of the ionic liquid, a few solids were also carried out. The solids were separated by filtration and weighed. Its mass was added to the total mass of the precipitate. Then, the precipitate was fully characterised: total solids, volatile solids, protein, carbohydrates, sugars and ashes were determined. Finally, a Fourier Transform Infrared (FTIR) spectroscopy was realised. The exact value of the cellulose recovered in the precipitate was calculated from the value of glucose obtained during the determination of sugars by strong hydrolysis.

2.5. Analytical characterization

The total solids and volatile solids of all phases were analysed according to standard methods 2540B (Rice et al., 2012), ash content in all phases was determined by the standard method 2540E (Rice et al., 2012). Moisture was calculated from the value of total solids by difference between the initial weight of sludge minus the weight of total solids.

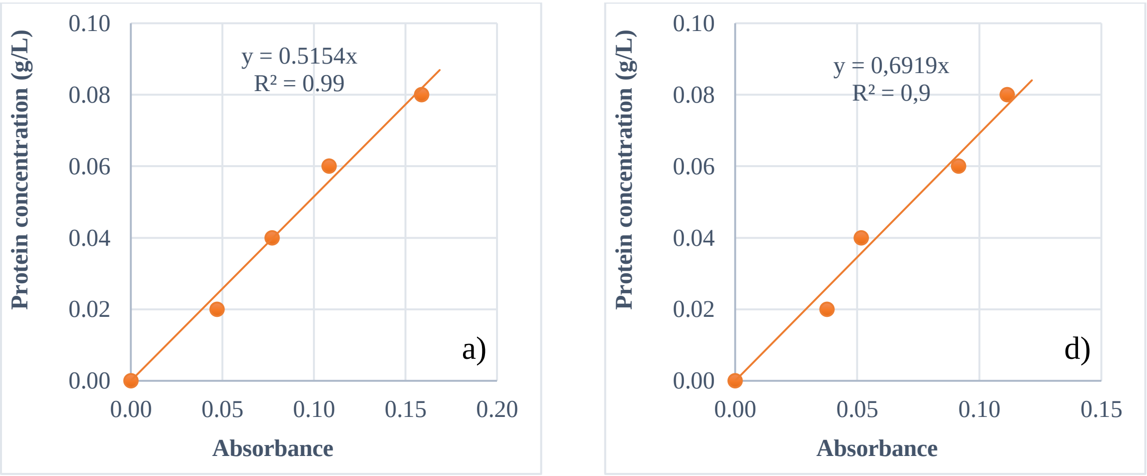
The lipids were determined following the standard method 5520E (Rice et al., 2012). 20 g of wet industrial paper mill sludge was placed in a porcelain evaporating dish and acidified to pH=2 by adding 0.3 ml of HCl. Then, the sludge was dewatered by adding 25 g of $MgSO_4 \cdot H_2O$ to avoid interferences of water. The samples were mixed until getting a homogeneous paste.

After that, they were crushed 3 times during the 30 min until they became a fine powder. Then, the samples were transferred into the cellulose Soxhlet extraction thimble and covered with a glass wool. The extraction was done in a Soxhlet extractor using hexane as a solvent at a velocity of 20 cycles/hour for 4 h. Subsequently, the hexane was evaporated through a rotary evaporation. The lipids were kept in a desiccator overnight to remove hexane and weighed the next day. The volumetric flasks were measured before and after the experiment to recuperate the lipid content. Mass of lipids was calculated by weighing and with the formula:

$$\mathrm{Lipids}(\%_{TS}) = \frac{W_{Lipids}(g)}{W_{TS}(g)} \qquad (1)$$

where W_{Lipids} was the weight of lipids after evaporation of hexane and W_{TS} was the initial weight of total solids of the 1 g sample.

The protein quantification was determined by the Lowry method using folin phenol reagent (Lowry et al., 1951). The samples were prepared following the corresponding dilutions. For the calibration curve, 0.5 g/L of Bovine serum albumin was used. The absorbance was measured using a spectrophotometer at 750 nm. For the determination of protein in the liquid phase in presence of ionic liquids, six calibration curves were realised, one for each type of ionic liquid and for each concentration of ionic liquid. The calibration curves are presented in Figure 3. The rest of the procedure was the same as the previous one. The amount of protein was calculated by the colorimetric method.



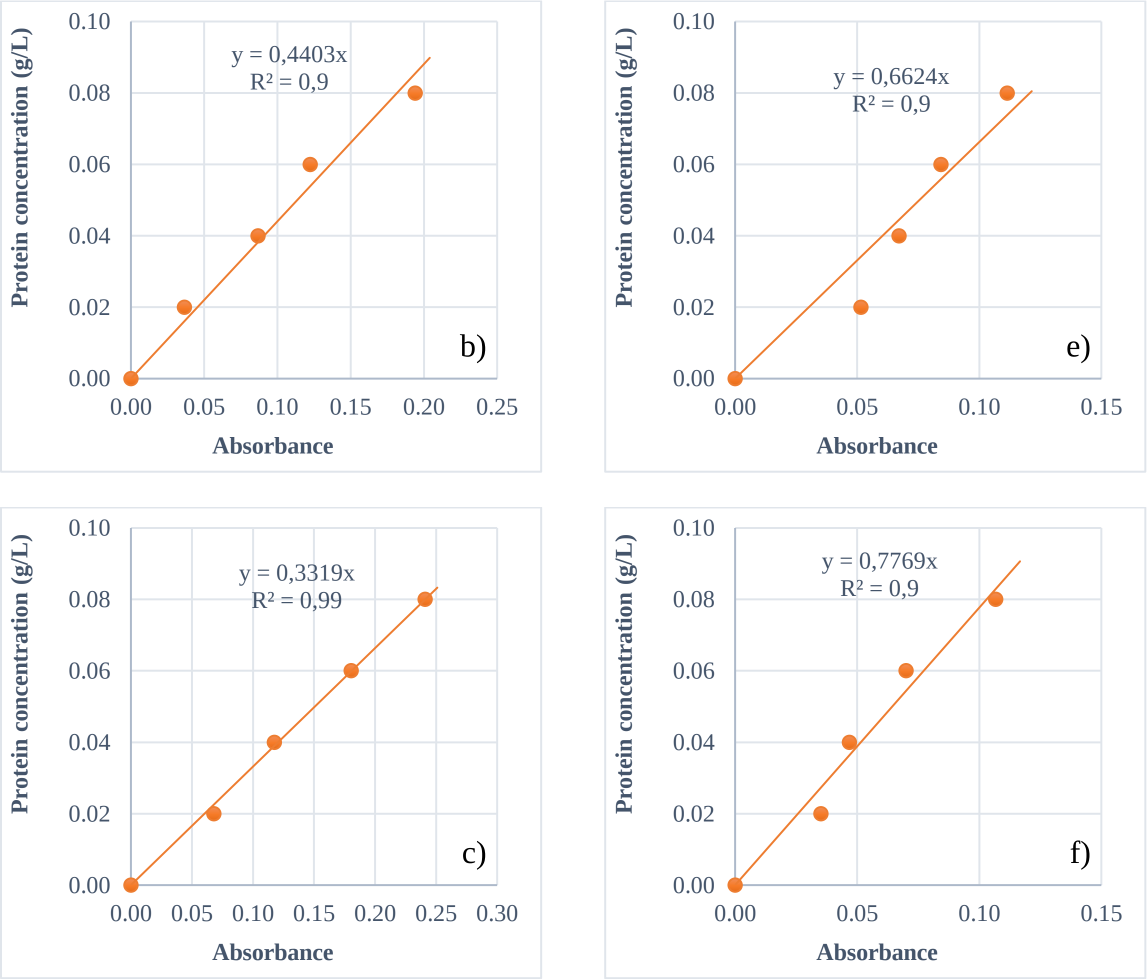


Figure 3. Determination of proteins in the aqueous liquid phase by Lowry method (Lowry et al., 1951). Calibration curves obtained in presence of the ionic liquid: a) Tetrakis 2 mL, b) Tetrakis 5 mL, c) Tetrakis 10 mL, d) Imidazolium 2 mL, e) Imidazolium 5 mL, f) Imidazolium 10 mL.

Total sugar, or carbohydrates determination, was performed according to phenol sulphuric method of Dubois (Dubois et al., 1956). 0.1 g/L of glucose was used to prepare the calibration curve. The samples were analysed through the spectrophotometer at 480 nm.

Cellulose quantification was performed according to the strong hydrolysis method with some modifications (Sambusti et al., 2016). For this, 300 mg of dried industrial paper mill sludge sample was weighted in a beaker with 3 ml of 72% of H_2SO_4 dissolution. At the beginning, the solids were crushed in the presence of the sulphuric acid and then, the solution was left stirring continuously at room temperature for 1 h. After that, distilled water was added to the solution in order to dilute the acid to 4%. Then, the beakers were covered with aluminium foil and placed in the autoclave at 120ºC for 1 h. After, the solutions were cooled down, filtered using glass microfiber filters (FILTER.LAB, MFV3090, size: Ø=90 mm). The filtrate part was additionally filtered with a filter syringe of 0.22 μm into a HPLC vial. The concentration of monomers was then determined by HPLC using the Agilent Application Note "Analysis of By-products in Fermentation Liquids Using n Agilent Hi-Plex H column" and with the Hi-Plex H column (ref. PL1170-6830). The amount of cellulose was determined by the equation:

$$\mathrm{Cellulose}(\%_{TS}) = \frac{\mathrm{Glu\cos e}(\%)}{1.11} \tag{2}$$

where the value of glucose was determined by HPLC.

The precipitate was analysed by FTIR spectroscopy to check the presence of carbohydrates. The 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. Figure 4 presents the spectres obtained with tetrakis ionic liquid.

Finally, the structural morphology of pure cellulose and the cellulose recovered by both ionic liquids were studied by Environmental surface electron microscopy (ESEM). The analysis was carried out by Quanta 600 equipment. The material was directly placed onto the sample holder, covered with gold and images were obtained at 15kV. Figure 5 presents ESEM images of pure and recovered cellulose.

3. Results and discussion

3.1. Characterisation of industrial paper mill sludge

The results of the characterisation of the industrial paper mill sludge are presented in Table 1. As it can be seen in the table, the sludge contains 53.1% of moisture. The total solids, 46.9%, are constituted essentially by ashes, 49.5, and volatile solids, 50.6%. In fact, only 23.2% of the sludge is organic matter. This industrial sludge differs a lot of the classical urban sludge, where organic matter values are near 60-80% and the ashes between 20-30% of the total solids. The high presence of ashes is due to the mixing of the two sludges, from membrane bioreactor and from the process with recovered paper where the deinking process produces a lot of ashes. The determination of lipids, protein and carbohydrates was realised with rehydrated sludge as analytical procedures require liquid samples. The hydration was done to dilute 10 times the total solids of the sludge. Table 1 also presents the characterisation of the rehydrated sludge. The same value of total solids after dilution was expected, but minor differences, 1%, were obtained from ashes and volatile solids. The value of carbohydrates obtained was near 42.3%, indicating a possible high content of cellulose in the sludge. The origin of the sludge, ie. paper industry, is the direct responsible of this high value. On the other hand, the value of proteins is about 5.0%, obtained essentially from the microorganisms present in the purge of the membrane bioreactor. Finally, the value of lipids is very small, 1.2%, contributed also by the microorganisms. In fact, the composition of this industrial paper mill sludge differs totally from an urban sludge. By these results, it turns out that the unique way to valorise the sludge well is by recovering the cellulose.

Table 1. Characterisation of industrial paper sludge and rehydrated paper sludge by conventional procedures.

	Industrial paper sludge	**Rehydrated paper sludge**
TS (%)	46.9 ± 1.1	4.7 ± 0.1
Moisture (%)	53.1 *	95.3 *
Ashes (%, w/w_{TS})	49.5 ± 0.2	50.5 ± 1.1
VS (%, w/w_{TS})	50.6 *	49.5 *
Lipids (%, w/w_{TS})	N.R.	1.2 % ± 0.3 %
Proteins (%, w/w_{TS})	N.R.	5.0 % ± 0.6 %.
Carbohydrates (%, w/w_{TS})	N.R.	42.3 % ± 0.7 %
Total	-	99.0 %

Values are mean, ±SD, n=3.

N.R.: not realised.

* By difference.

3.2. Procedure to recover cellulose: Solubilisation of cellulose

The process of recovery of cellulose from industrial paper mill sludge was performed using a similar method conducted for the extraction of lipids from sewage sludge (Olkiewicz et al., 2015a). All the steps of the process of recovery of cellulose is depicted in Figure 2. The first step of the process was the rehydration of the sludge with the objective of allowing its full characterisation and to facilitate the action of the ionic liquids. During the rehydration, total solids were diluted from 46.9% to 4.7%. Then, imidazolium and tetrakis ionic liquids were added. Three different volumes were utilised for both ionic liquids: 2, 5 and 10 mL. The temperature of process was fixed at 100ºC and the solubilisation of the cellulose was performed during 24 h. All these values were adopted from previous studies (Olkiewicz et al., 2015a, b), where higher temperature and time provided the best results. In the same way, the increase of the sludge:IL ratio provoked an increase in the recovery of cellulose. Since in the industrial paper mill sludge it is expected that the quantity of cellulose will be higher, the sludge:IL ratio was increased until to 1 g_{TS}:10 mL_{IL}. Then methanol was added to precipitate the cellulose and three phases were obtained; organic phase (not very visible by its small quantity), aqueous phase (containing ionic liquid, water, methanol, part of the protein and part of the ashes) and precipitated phase (containing all cellulose and part of protein and ashes).

3.3. Separation of the lipids

The first step was to separate the lipids dissolved in the organic phase. Said lipids were dissolved by adding hexane to the mixture and separating them. Then, the organic phase was dried and the hexane was eliminated with a rotary evaporator. Finally, lipids were weighed and quantified. Table 2 presents the values obtained for both ionic liquids for the three ratios. As it can be seen in the table, the values of lipids are comparable in all experiments, differences can be attributed to experimental errors. The value of lipids present in the paper mill sludge is very small, 1.2% in average in total solids basis. The value is comparable to the one obtained by the classical Soxhlet method, confirming that this process with ionic liquids is able to separate well lipids from sludge. This value is smaller than the obtained in sludge from WWTP where, the value for secondary sludge was 7.7% (Olkiewicz et al., 2014). The level of lipids is as expected since in the process of manufacturing cellulose tissue, oils, greases or fats are not used, and the only source of lipids are the purged microorganisms coming from the membrane bioreactor.

3.4. Separation and characterisation of the aqueous liquid phase

After the separation of the lipids, the mixture was centrifuged. The supernatant was separated and cleaned with methanol, and then methanol was eliminated with a rotary evaporator. Finally, the liquid phase, containing ionic liquid, water and the rest of the paper mill sludge, was fully characterised.

Table 3 presents the values of total solids (TS), volatile solids (VS) and ashes present in the liquid phase after the extraction with both ionic liquids. As can it be seen in the table, for both ionic liquids, the value of the total solids increases with the increase in the volume of ionic liquid used for the separation. This means, and as expected, that the temperature of 105ºC is not enough to eliminate the ionic liquids from the samples and they remain mixed with the solids. The most interesting values that the table presents are those of the ashes. A very different behaviour can be observed for both ionic liquids. In the case of tetrakis, the amount of ash is an order of magnitude higher than in the case of imidazolium. This suggests that the tetrakis allows to better separate the cellulose from the ashes. The increase in the amount of IL used allows more ash to be removed, however, the 1 g_{TS}:10 mL_{IL} ratio does not show improvement. This difference in behaviour is very important and will have an influence during the selection or design of ionic liquids to separate cellulose.

Table 2. Weight of lipids extracted during the process.

	Lipids (%) [a]	
Ionic liquid	"tetrakis"	"imidazolium"
Ratio sludge:IL	$C_4H_{12}ClO_4P$	$C_9H_{18}N_2O_4S$
1 g: 2 mL	1.1	1.3
1 g: 5 mL	1.2	1.3
1 g: 10 mL	1.0	1.2

Experimental conditions: 24 h of reaction, 100ºC of temperature.

[a] 100 x Weight of lipids / Weight of total solids

Table 4 presents the values of the protein in the liquid phase. As it can be seen in the table, the amount of protein collected in the liquid phase is quite different for both ionic liquids. Imidazolium is capable of retaining a greater amount of protein than tetrakis. However, the values say that imidazolium is not able to retain all the protein present in the sludge sample. In both cases, the separation of the cellulose protein is not complete.

Table 3. Total solids (TS), volatile solids (VS) and ashes in the liquid phase.

	Solids in the liquid phase							
Ionic liquid	"tetrakis" $C_4H_{12}ClO_4P$				"imidazolium" $C_9H_{18}N_2O_4S$			
Ratio sludge:IL	TS [a]	Ashes [b]	Ashes [c]	VS [d]	TS [a]	Ashes [b]	Ashes [c]	VS [d]
1 g: 2 mL	8.4	28.5	2.4	71.5	12.5	1.9	0.2	98.1
1 g: 5 mL	21.7	19.8	4.3	80.2	36.4	0.8	0.3	99.2
1 g: 10 mL	29.6	11.6	3.4	88.4	52.0	0.5	0.3	99.4

Experimental conditions: 24 h of reaction, 100ºC of temperature.

[a] (in %) 100 x Weight of solids / Weight of liquid phase sample.

[b] (in %) 100 x Weight of ashes / Weight of solids.

[c] (in %) 100 x % of ashes x % of total solids.

[d] (in %) By difference, 100 – (%) of ashes.

Table 4. Protein in the liquid phase.

	Protein in the liquid phase (%)	
Ionic liquid	"tetrakis"	"imidazolium"
Ratio sludge:IL	$C_4H_{12}ClO_4P$	$C_9H_{18}N_2O_4S$
1 g: 2 mL	1.2 ± 0.1	3.8 ± 0.2
1 g: 5 mL	2.1 ± 0.2	3.7 ± 0.2
1 g: 10 mL	1.9 ± 0.1	3.4 ± 0.4

Experimental conditions: 24 h of reaction, 100ºC of temperature.

Values are mean, ±SD, n=3.

Table 5 presents the values of the carbohydrates (cellulose) in the liquid phase. The presence of a small amount of carbohydrates has been detected in the liquid phase after separation with both ionic liquids. This shows that although the precipitation separation of cellulose has a very important yield, it is not complete. Therefore, it will be necessary in the future to optimize the design of ionic liquids in order to carry out a complete precipitation of cellulose.

Table 5. Carbohydrates in the total solids (TS) of the liquid phase.

	Carbohydrates in the liquid phase (%)	
Ionic liquid	“tetrakis”	“imidazolium”
Ratio sludge:IL	$C_4H_{12}ClO_4P$	$C_9H_{18}N_2O_4S$
1 g: 2 mL	3.1	1.2
1 g: 5 mL	2.8	1.9
1 g: 10 mL	2.8	1.2

Experimental conditions: 24 h of reaction, 100ºC of temperature.

3.5. Characterisation of the precipitated phase

As mentioned previously, the precipitated phase was obtained by addition of methanol to the mixture containing dissolved cellulose. The precipitate containing cellulose and part of the proteins and ashes was separated by centrifugation at 6000 rpm during 10 min. The precipitate was cleaned with methanol and centrifuged again in the same conditions. The precipitate was fully characterised, determining total solids, volatile solids, ashes, protein, carbohydrates and sugars. Finally, it was characterised by FTIR and ESEM.

Table 6 presents the values of the weight of the precipitate recovered after treatment. The behaviour of recovery is similar for both ionic liquids. The increase of the ratio sludge:IL from 1 g_{TS}:2 mL_{IL} to 1 g_{TS}:5 mL_{IL} decreased the weight of precipitate produced. But a further increase of the ratio from 1 g_{TS}:5 mL_{IL} to 1 g_{TS}:10 mL_{IL} increased the weight of the precipitate. This behaviour can only be explained by a different composition or non-homogeneity of the samples when they were extracted. On the other side, it can be noticed that the weight obtained with imidazolium ionic liquid is higher than with tetrakis, the increment was between 30 and 35%. The values of the total solids presented in Table 7 show that the precipitate has generally been very well separated since the moisture content is usually always less than 2%. Only in the case of the ionic liquid imidazolium and with a ratio of 1 g_{TS}:5 mL_{IL} attained 3%. The values of volatile solids, also presented in Table 7, have more interest. In fact, each ionic liquid presents completely different values, in the case of tetrakis ionic liquid the volatile solids correspond to

76-77% while in the case of imidazolium they only represent 53-55%. In these cases, the sludge: IL ratio do not affect the final result. It can be suspected that in the future a smaller ratio could be used. What are remarkable are the differences in the amount~~s~~ of ashes present in the precipitates. The amount of ash present in the precipitate with the imidazolium ionic liquid is double in all studied ratios, which means that the tetrakis allows a better separation of the ashes.

Table 6. Weight of precipitated solids after extraction process.

	Precipitate (%) *	
Ionic liquid	"tetrakis"	"imidazolium"
Ratio sludge:IL	$C_4H_{12}ClO_4P$	$C_9H_{18}N_2O_4S$
1 g: 2 mL	64.5	87.8
1 g: 5 mL	60.4	83.2
1 g: 10 mL	67.5	87.7

Experimental conditions: 24 h of reaction, 100ºC of temperature.

* Weight of precipitated solids / Weight of total solids.

Table 7. Total solids (TS), ashes and volatile solids (VS) in the precipitated phase.

	Solids in the precipitated phase (%)					
Ionic liquid	"tetrakis" $C_4H_{12}ClO_4P$			"imidazolium" $C_9H_{18}N_2O_4S$		
Ratio sludge:IL	TS [a]	Ashes [b]	VS [c]	TS [a]	Ashes [b]	VS [c]
1 g: 2 mL	98.9	23.4	76.6	99.5	44.6	55.4
1 g: 5 mL	98.3	22.5	77.5	96.9	44.1	55.9
1 g: 10 mL	98.3	23.5	76.5	99.1	47.0	53.0

Experimental conditions: 24 h of reaction, 100ºC of temperature.

[a] Weight of total solids x 100 / Weight of precipitate.

[b] Weight of ashes x 100 / Weight of total solids.

[c] By difference, Weight of total solids – Weight of ashes.

Table 8. Proteins in the precipitated solids.

	Precipitate (%) *	
Ionic liquid	“tetrakis”	“imidazolium”
Ratio sludge:IL	$C_4H_{12}ClO_4P$	$C_9H_{18}N_2O_4S$
1 g: 2 mL	4.0	1.4
1 g: 5 mL	3.1	1.8
1 g: 10 mL	3.3	1.6

Experimental conditions: 24 h of reaction, 100ºC of temperature.

* Weight of protein / Weight of total solids.

Table 9. Cellulose and yield of recovered cellulose calculated from the experimental mass balance.

Ionic liquid	**“tetrakis” $C_4H_{12}ClO_4P$**		**“imidazolium” $C_9H_{18}N_2O_4S$**	
Ratio sludge:IL	Cellulose in the precipitated phase (%) [a]	Yield of recovered cellulose (%) [b]	Cellulose in the precipitated phase (%) [a]	Yield of recovered cellulose (%) [b]
1 g: 2 mL	45.4	104.9	47.2	109.1
1 g: 5 mL	43.7	100.9	44.7	103.2
1 g: 10 mL	48.3	111.6	44.9	103.6
Average	45.8 ± 2.3	105.8 ± 5.4	45.6± 1.4	105.3± 3.27

Experimental conditions: 24 h of reaction, 100ºC of temperature.

Values with deviation are mean, ±SD, n=3.

[a] By difference (Weight of precipitate × % of volatile solids/100) – weight of protein.

[b] 100 × Cellulose in the precipitated phase / 43.3. Table 1: 49.5 (volatile solids) – 5.0 (protein) – 1.2 (lipids) = 43.3

On the contrary, the protein content in the precipitate is higher when the ionic liquid tetrakis is used. As it can be seen in Table 8, the amount of proteins present in the precipitate can reach 4%, while in the case of imidazolium it never exceeds 2%. In short, the behaviour of both ionic liquids is different; tetrakis ionic liquid is able to separate ashes better, and protein worse, than imidazolium.

The values of recovered cellulose and its yield, both calculated from experimental mass balance, are presented in Table 9. The value of cellulose was calculated by Eq. (3):

$$\text{Cellulose}(\%) = \frac{(\%\text{of Precipitate})\times(\%\text{of VS})}{100} - (\%\text{of Protein in the precipitate}) \qquad (3)$$

where the (% of Precipitate) are the values presented in Table 6, (% of VS) are the values presented in Table 7, and (% of protein in the precipitate) are the values presented in Table 8. The value of the yield of cellulose was calculated by Eq. (4):

$$\text{Yield of cellulose}(\%_{TS}) = \frac{\text{Cellulose}(\%)}{(\%\text{of VS})_{bt} - (\%\text{of Protein})_{bt} - (\%\text{of Lipids})_{bt}} \qquad (4)$$

where cellulose was calculated by relation (5), $(\%\text{ of VS})_{bt}$ is the value of volatile solids before treatment (bt) presented in Table 1, $(\%\text{ of Protein})_{bt}$ is the value of protein presented in Table 1 and $(\%\text{ of Lipids})_{bt}$ is the value of lipids presented in Table 1. As it can be seen in the table, the values of cellulose are very similar for all experiments realised. There are not notable differences between the values obtained with both ionic liquids, the average values being equal, differences must be found elsewhere. The yields of cellulose obtained are over 100% caused by the mass of cellulose obtained. Taking into account experimental errors, it is remarkable that the mass balance is close to 100 for both ionic liquids.

Table 10 presents the values of carbohydrates in the precipitate, determined by strong hydrolysis of the precipitate followed by HPLC determination. The amount of carbohydrates is the sum of the xylose and glucose quantified by HPLC. As it can be seen in the table, the values of carbohydrates obtained by hydrolysis for imidazolium ionic liquid is higher than expected if compared with values in Table 9. In the case of the tetrakis ionic liquid, there is an important difference between the two quantities included between 6 and 15%. The origin of this non identified organic matter has not been yet determined. However, some authors suspect the possible presence of lignin or humin like composts in the sludge, as it is the case with food wastes (di Bitonto et al., 2018). For the weight of cellulose quantified by HPLC, the values are obtained from the concentration of glucose in the hydrolysed sample divided by the factor 1.11

(Sambusti et al., 2016). The values of cellulose obtained are very close for each ratio for both ionic liquids. However, the values obtained for tetrakis are 15% higher than for the imidazolium, 53% instead of 38%. This contradicts the results of the experimental mass balance, where both ionic liquids where able to recover the same quantity of cellulose. One explanation for this is that the strong acidic hydrolysis affected the structure of sugars and protein contained in the precipitate. On the other hand, it is also possible that the ashes contained in both precipitates had also a big influence during hydrolysis. This will be investigated in detail in the future. As mentioned before, it should also be noted that the sludge:IL ratio does not significantly affect the cellulose recovery. Except for the case of the ratio 1 g_{TS}:5 mL_{IL} for both ionic liquids where there is a decrease in the amount of cellulose of 8%. This phenomenon has already been discussed by presenting the results of Table 6. This decrease only affects especially the amount of cellulose and not the other substances present in the precipitate.

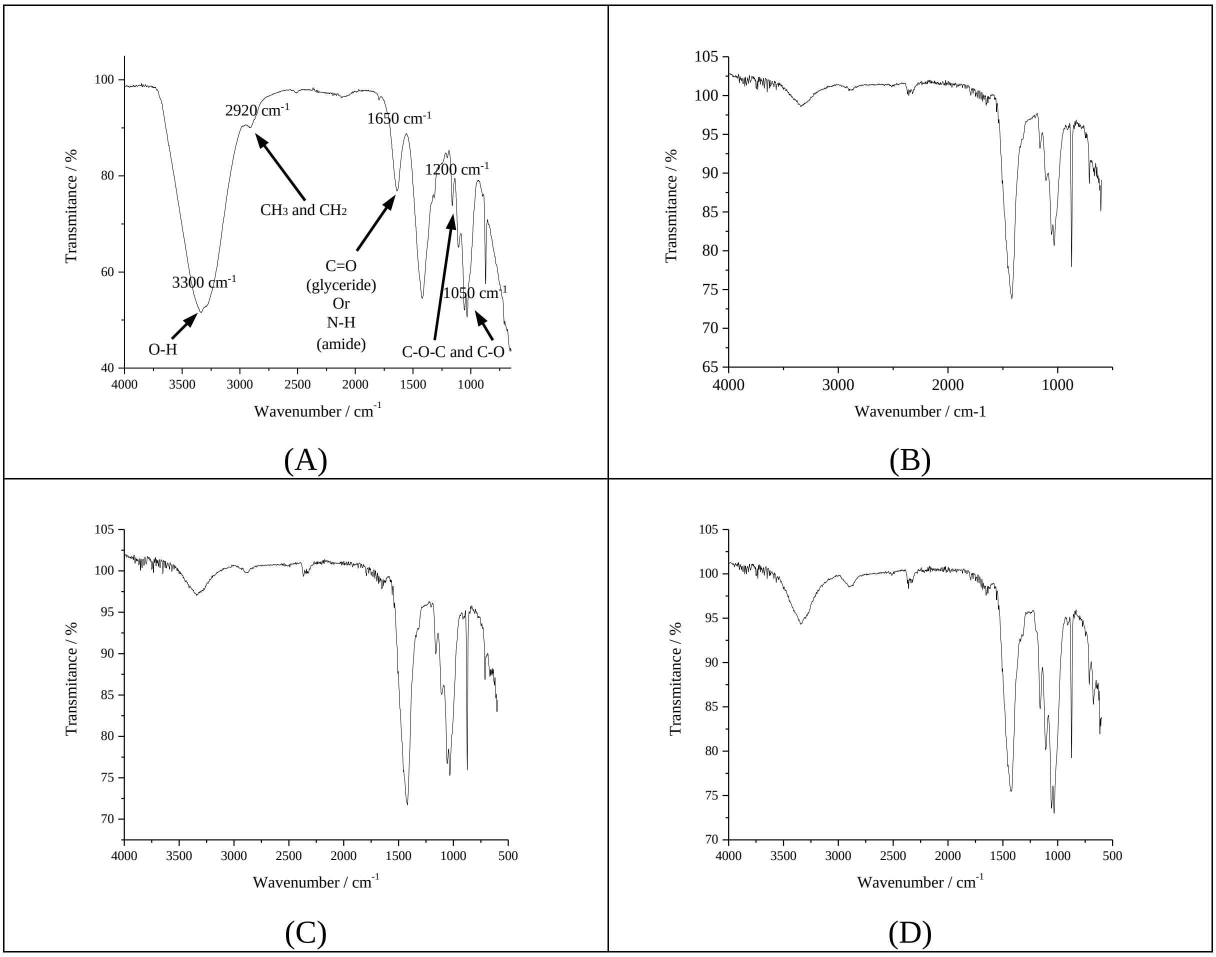
Table 10. Carbohydrates and cellulose in the precipitated phase calculated by HPLC after strong hydrolysis.

Ionic liquid	**“tetrakis”** **$C_4H_{12}ClO_4P$**		**“imidazolium”** **$C_9H_{18}N_2O_4S$**	
Ratio sludge:IL	Carbohydrates in the precipitated phase (%) [a]	Cellulose in the precipitated phase (%) [b]	Carbohydrates in the precipitated phase (%) [a]	Cellulose in the precipitated phase (%) [b]
1 g: 2 mL	66.1	53.7	49.2	38.5
1 g: 5 mL	60.7	49.4	45.9	35.9
1 g: 10 mL	64.7	52.8	49.5	38.8

Experimental conditions: 24 h of reaction, 100ºC of temperature.

[a] By strong hydrolysis of precipitate and HPLC.

[b] Measured by HPLC using a factor conversion of 1.11 with the value of the glucose detected and determined.



(A) (B)

(C) (D)

Figure 4. FTIR spectra of the dried sludge (A) and precipitated phase after extraction with imidazolium ionic liquid, 24 hours and 100ºC of temperature: 1 g: 2 mL ratio (B); 1 g: 5 mL ratio (C); 1 g: 10 mL ratio (D).

Figure 4 presents the Fourier Transform Infrared (FTIR) spectres of dried sludge as received and precipitate after treatment with imidazolium ionic liquid at the three sludge:IL ratios utilised. The spectra of dried sludge (spectra A) present some characteristic absorbances in different frequency regions attributed to specific groups: ~3300 cm^{-1} of O-H groups; ~2920 cm^{-1} of CH_3 and CH_2 stretching vibration in lipid acyl chain; ~1650 cm^{-1} of C=O groups in lipid esters (glycerides) or N-H groups for peptide amide groups of proteins; ~1200 cm^{-1} of C-O-C groups, stretching vibration particularly associated with cellulose; ~1050 cm^{-1} of C-O groups normally associated to carbohydrates (Olkiewicz et al., 2015a). The peak at 1650 cm^{-1} should rather be attributed to the presence of proteins and not to lipids since in the composition of the sludge the lipids represent only 1.2% of the total solids while the protein represents 5.0%. The peaks attributed to C-O-C and C-O confirm the presence of cellulose in the sludge. The comparison with a FTIR spectra of pure cellulose will corroborate this affirmation (Olkiewicz et al., 2015a). The three spectra of the precipitates after separation with the ionic liquid (spectra B, C and D) do not show large differences between them. However, with respect to the spectrum of the dry sludge, differences can be appreciated. The peak intensity of the O-H group has decreased in large proportions. The characteristic peaks of carbohydrates and cellulose (1200 and 1050 cm^{-1}) are maintained with the same intensities, which would mean that almost all of the cellulose has been recovered. But the most important thing is that the peak at 1650 cm^{-1} of lipids has practically disappeared, meaning that the precipitate no longer contains large amounts of protein neither lipids. This fact confirms the results presented in Table 8.

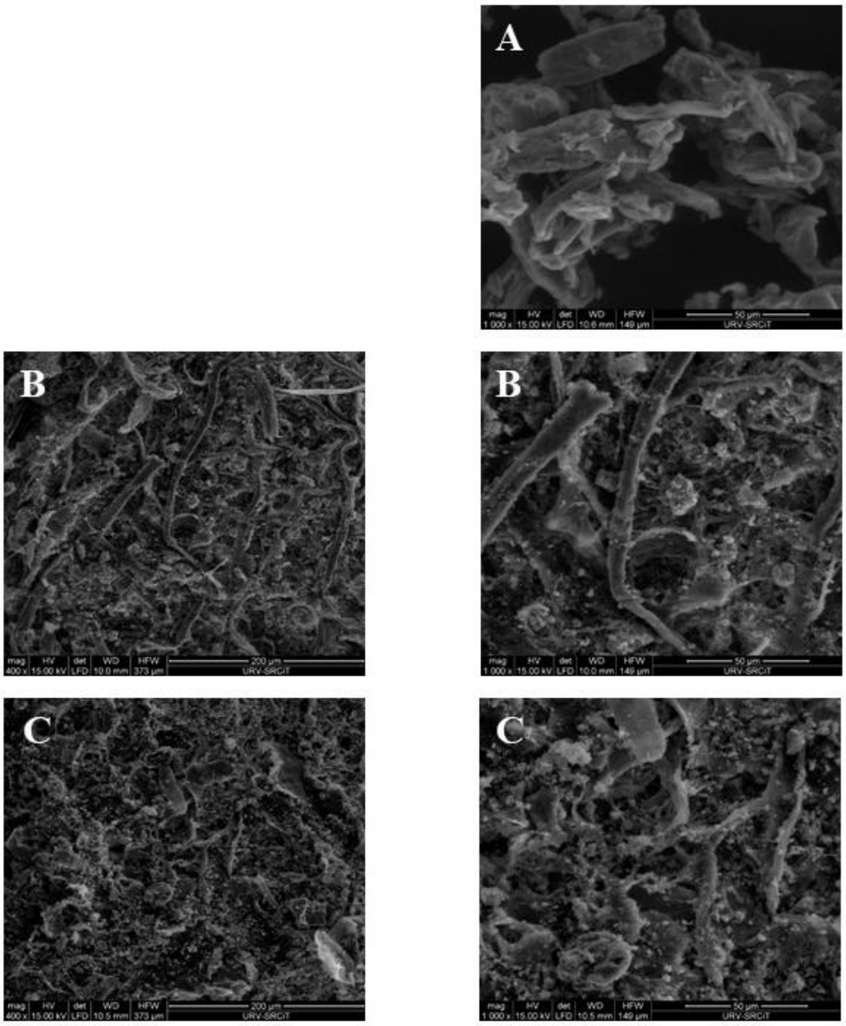


Figure 5. ESEM Images of commercial cellulose (A), cellulose recovered with Tetrakis IL (B; 400× and 1000×), cellulose recovered with Imidazolium IL (C; 400× and 1000×).

Figure 5 shows images of ESEM carried out on pure cellulose (A) and cellulose recovered by tetrakis (B) and imidazolium (C) ionic liquids. On the left, pictures are presented at magnitude

400x and on the right at 1000x. It can be observed that all images present the bundles of fibres. As pure cellulose, sigmacell cellulose 20 µm was used, hence on the picture we can see short fibres. In a case of cellulose recovered by ionic liquids it can be noticed that tetrakis leaves long fibres, organized separately whereas imidazolium makes the fibres shorter and surrounded by other components presented in precipitate, mainly by ashes. Morphology of the precipitates in both examples confirm that what was obtained was cellulose.

In short, the two ionic liquids have made it possible to separate cellulose with a certain degree of purity. The main differences can be summarized in three important aspects: tetrakis allows to obtain a greater amount of cellulose; it also allows the amount of ash to be much lower in the precipitate; imidazolium allows the amount of protein to be much lower in the precipitate. As the results show, there is still a lot of work to be done in designing ionic liquids that are capable of separating cellulose with high purity in a single operation. The enormous volumes of wastewater and sludge generated by the paper industry make the process worthwhile.

4. Conclusions

It has been demonstrated that ionic liquids are able to recover cellulose from rehydrated dried sludge provided by an industrial paper company. Both ionic liquids, 1-butyl-3-methyl imidazolium methyl sulphate and tetrakis (hydroxymethyl) phosphonium chloride, present great recoveries of cellulose. But some differences are noticed in the behaviour of the processes with both ionic liquids. Tetrakis allows a less content of ashes in the precipitated phase. On the other hand, imidazolium ionic liquid lets a minor quantity of the proteins in the precipitated phase. Recovery process must be optimised by the design of new ionic liquids having the combined performances of tetrakis and imidazolium ionic liquids. Operation conditions, 100ºC of temperature and 24 h of process, do not allow to already scale-up the process, but the optimisation of these conditions have to be done in parallel to the design of ionic liquids. Finally, this was technical proof of concept that must be optimised before being applicable to the paper industry, generating of large amounts of wastewater and sludge.

Acknowledgements

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Chapter 4

Moving municipal WWTP towards circular economy: cellulose recovery from primary sludge with ionic liquid [1]

ABSTRACT

Tetrakis (hydroxymethyl) phosponium chloride ionic liquid was evaluated to recover carbohydrates, especially cellulose, from municipal wastewater treatment plant (WWTP) wet primary sludge (96% water content). Different process operative conditions were evaluated: temperature (35, 60 and 80 ℃), time (6, 13 and 20 h) and sludge weight:ionic liquid volume ratio (1g $_{\mathrm{TS\ of\ SLUDGE}}$: 7, 12 and 17 mL). A three levels, three factors central design was applied to optimize the procedure of recovery of the cellulose. Precipitated solids were analysed to quantify the content of cellulose, hemicellulose, lignin, proteins and ashes. The ionic liquid showed good performances in the recovery of cellulose. The average amount of carbohydrates recovered using the ionic liquid method was comparable with the amount of carbohydrates measured by the Dubois method in the raw sludge. All cellulose was recovered in the precipitated solid, but also part of the protein and ashes. According to the statistical tests, two interactions were found: between temperature and sludge:IL ratio for recovery of hemicellulose; between temperature and time for recovery of proteins. Experimental design was not useful to find optimized conditions of the process. Experiments realised with central values (60ºC; 13 h; 1:12 mL) gave very good results, allowing the recovery of high quantities of cellulose with the best removal of ashes but maintaining high quantities of proteins in the precipitate. For these reasons central values can be considered optimal reaction conditions. The design of novel ionic liquids will be necessary to fully purify the cellulose in a single dissolution step.

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[1] Glińska K., Stüber F., Fabregat A., Giralt J., Font J., Mateo-Sanz J.M., Torrens E., Bengoa C. Moving municipal WWTP towards circular economy: cellulose recovery from primary sludge with ionic liquid. *Resources, Conservation & Recycling* 154 (2020) 104626.

1. Introduction

The management of municipal wastewater from human activity is a serious problem in many of the urban and industrial regions. The sludge produced during wastewater treatment consists essentially of organic and inorganic matter that precipitates during the treatment processes. This sludge cannot longer be used as fertilizers in several countries, increasing the problem of their management (Vadenbo et al., 2014). On the other hand, management strategies should fulfil the main concept of European Commission “reduce, reuse, recycle”, the eco-innovation trend in waste management hierarchy in European countries (Directive 2008/98/EC). A full recycling of organic matter and reduction of the potential risk (pollutants) should be allowed thanks to the management strategies (Kacprzak et al., 2017). For this reason, the municipal wastewater treatment sector is positioning itself as a major player in the shift towards a circular economy. This requires a change of approach, since the main objective assigned to the WWTP is the treatment of wastewater. The transformation from wastewater treatment plants to water resources recovery facilities completely changes the objective, from water cleaning to resource recovery. This new approach allows to give a second life to the resources present in the wastewater, and its recovery and valorisation becomes one of its main objectives (Sfez et al., 2019) which is in accordance with European Commission (2011): “if waste is to become a resource to be fed back into the economy as a raw material, then, much higher priority need to be given to reuse and recycling”. From the environmental point of view, reutilization of sewage sludge reduces greenhouse gas emissions, fossil fuel use and improves soil conditions. From the economical point of view, reutilization of sewage sludge compensates the costs related to traditional waste treatment methods and decreases energy costs from the usage of biogas and biofuels.

A typical municipal WWTP produces three main types of sludge, primary, secondary and digested (Tyagi and Lo, 2013). The secondary sludge is a complex biomass, composed mainly of microbial cells and suspended solids, produced during the aerobic biological digestion of organic matter of primary wastewater collected in the secondary settler. During sludge digestion, when digested sludge is formed, organic solids are decomposed into stable substances. Digestion reduces the total mass of solids and odors, destroys pathogens, and makes it easier to dewater or dry the sludge. Finally, and the most interesting, the primary sludge, is a combination of floating grease and solids. These solids are valuables organic components as protein, lipids, carbohydrates and finally, ashes.

In the recent past, authors, following rules of circular economy, were able to recover enzymes from municipal secondary sludge (Nabarlatz et al., 2012) and lipids from municipal primary sludge, to convert them to biodiesel (Olkiewicz et al., 2015a; Olkiewicz et al., 2015b).

Currently, lignocellulosic biomass is very poorly valued, despite having a high potential for the production of molecules with high added value (Paul and Dutta, 2018), nanocellulose (Pires et al., 2019), syngas and highly functionalized aromatic compounds (Gazi 2019) or bioethanol (Chohan et al., 2020). For this reason, continuing with the recovery and valorization of valuable organic components, authors are now focusing in carbohydrates present in primary sludge.

Among the components of primary sludge, carbohydrates are the major components, around 26.2 ± 2.6 % based on dry matter (Olkiewicz et al., 2015a). These carbohydrates come essentially from the discharge of toilet paper (Honda et al., 2002). According to a report of Foundation for Applied Water Research (STOWA, 2012), citizen send between 10 kg to 14 kg of toilet paper into the sewage system each year. These values represent 30% to 50% of the suspended solids of the influent. Then, cellulose can be considered as the major component of carbohydrates in primary sludge. Cellulose recovered from the sludge can be used in packaging industry, for bioethanol production or as a substrate to obtain a value-added chemical.

Cellulose is one of the organic matter components worth to recycle because of its main properties: biocompatibility, biodegradability, thermal and chemical stability (Mohd et al., 2017). Cellulose can be processed into value added molecules, building bio-blocks, bioplastics and flocculants. The main problem in the cellulose processing is its insolubility in water and in common organic solvents. Cellulose has a rigid crystalline supramolecular structure built with strong inter- and intramolecular hydrogen bonding and hydrophobic interactions (Bodachivskyi et al., 2018; Gupta et al., 2018). All these characteristics make the recovery of cellulose difficult.

An alternative to dissolving cellulose are the ionic liquids (IL/ILs) (Swatloski et al., 2002). ILs are salts with melting point temperatures below 100ºC. Their structure contains an organic bulky cation and an organic or inorganic anion (Wahlstrom and Suurnakki, 2015). ILs are used as green replacement for harmful volatile organic solvents due to their non-volatile character, excellent chemical and thermal stability. They stand out potential recoverability, design possibilities, and ease of separation of the products from reactants (Plechkova and Seddon, 2008).

Ionic liquids are suitable for cellulose dissolution because they disrupt and break intermolecular hydrogen bonding network of cellulose, significantly contributing to the dissolution process (Mohd et al., 2017; Uto et al., 2018). Ionic liquids commonly used for cellulose dissolution are

1-butyl-3-methylimidazolium chloride (BMIMCl), 1-allyl-3-methylimidazolium chloride (AMIMCl), 1-ethyl-3-methylimidazolium acetate (C_2MIMOAc) and 3-methyl-N-butylpyridinium chloride (C_4mpyCl) (Wang et al., 2012; Mohd et al., 2017). After dissolution, the cellulose can be precipitated by the addition of water, or other precipitating solution including ethanol, methanol, acetone, acetonitrile (Wang et al., 2012). These processes have been studied in past years (Swatloski et al., 2002; Pinkert et al., 2009; Wang et al., 2014). But, this kind of process was not really used with real municipal WWTP wet primary sludge.

The extraction of lipids from primary sludge using ionic liquids to produce biodiesel was realized in a previous work (Olkiewicz et al., 2015a). Phosphonium ionic liquid ($[P(CH_2OH)_4]Cl$) was also used in this work. The IL was suitable for the extraction of lipids, but also for the recovery of other valuable materials as cellulose and proteins (Olkiewicz et al., 2015a). Then, the purpose of this study, following previous work, is to optimize the use of ionic liquid~~s~~ to recover the cellulose from a municipal primary sludge. The ionic liquid was chosen because is one of the cheapest currently commercially available and as it was written above, it was already used for the recovery of organic components successfully. To the best of our knowledge, it is the first time that the optimization of the recovery of organic matter from wet primary sludge with tetrakis (hydroxymethyl) phosphonium chloride ionic liquid has been done. For this, three levels, three factors central design was used to optimize operative conditions of the process for a lab scale. Effects of temperature, time of dissolution and ratio between sludge and ionic liquid were evaluated in order to find the optimum conditions of the process.

2. Materials and methods

2.1. Reagents

Tetrakis (hydroxymethyl) phosphonium chloride (hydrated ionic liquid, 80% in water) $[P(CH_2OH)_4]Cl$ (ref: 404861) was supplied by Sigma-Aldrich. Solvents, methanol (ref: 32213) and hexane (ref: 15671), high performance chromatography grade, were provided by Sigma-Aldrich. Reagents, sulphuric acid 95.0-97.0% (ref: 30743), bovine serum albumin (BSA) (ref: A9647), sodium hydroxide (ref: S5881), sodium carbonate (ref: 222321), potassium sodium tartrate tetrahydrate (ref: 217255), copper (II) sulphate pentahydrate (ref: 209198), Folin&Ciocalteu's phenol reagent (ref: F9252), magnesium sulphate monohydrate (ref: 434183), anhydrous sodium sulphate (ref: 239313), and fuming hydrochloric acid (ref: 84418), high analytical reagent grade, were supplied by Sigma - Aldrich. Glucose monohydrate (ref: 49160) was purchased from Fluka and phenol crystalline (ref: 144852.1211) was provided by PanReacAppliChem. Sugar monomers xylose (ref: X3877), arabinose (ref: A3256), glucose (ref: G8270) and sugar oligomer cellobiose (ref: C7252) with purity ≥ 99% were supplied by Sigma-Aldrich.

2.2. Sludge collection and managing

The primary sludge was collected from the Wastewater Treatment Plant (WWTP) in Reus, Spain. The primary sludge was sampled after partial gravity thickening. Wet sludge (96% water content) was stored in a fridge at 4 ºC right after recollection. Sludge for characterization and experimentation were used as received.

2.3. Characterization of primary sludge by conventional methods

A full characterization of each batch of primary sludge used to recover the cellulose was realized in triplicate. Total solids (TS), volatile solids (VS) and ash content were determined according to standard methods 2540B and 2540E respectively (Rice et al., 2012). Protein content was determined according to Lowry method (Lowry et al., 1951). The protein solubilization in the sludge samples was carried out by heating the samples with 2 M sodium hydroxide at 100ºC for 10 min. The absorbance was measured at 750 nm. Total carbohydrate amount was quantified by phenol-sulfuric acid Dubois method (Dubois et al., 1956). Briefly, 0.05 mL of 80% phenol solution was added to 2 mL of diluted sludge sample in a glass tube. Then, 5 mL concentrated sulphuric acid was quickly added, directly over the liquid surface. The tubes were kept under room temperature for 10 minutes and then placed into a thermostatic bath at 30 ℃ another 15 minutes. The absorbance was measured at 480 nm.

Finally, extraction of lipid was carried out in a Soxhlet apparatus using hexane as a solvent according to standard method 5520E (Rice et al., 2012). All results of the full characterization of primary sludge are presented in Table 1.

Table 1. Primary sludge composition obtained by conventional methods.

TS [a]	**VS** [a]	**Lipids** [b]	**Carbohydrates** [b]	**Proteins** [b]	**Ashes** [b]	**Total** [c]
3.9±0.1	3.3±0.2	19.6±0.6	31.3±0.1	27.7±0.1	16.0±0.1	94.6±0.9
4.2±1.2 [d]	3.3±0.9 [d]	27.2±0.4 [e]	26.2±2.6 [e]	24.2±1.4 [e]	20.1±0.4 [e]	97.7±4.8

Values are means ± SD, n=3

[a] 100 x mass (g) / mass wet primary sludge (g)

[b] 100 x mass (g) / mass total solids (g)

[c] Lipids + Carbohydrates + Proteins + Ashes

[d] Olkiewicz et al., 2015b

[e] Olkiewicz et al., 2015a

2.4. Experimental procedure

Figure 1 shows the procedure of recovery of carbohydrates from municipal wet primary sludge using ionic liquids. As indicated in the scheme, 1 g of TS of wet primary sludge and a certain volume of ionic liquid tetrakis (hydroxymethyl) phosphonium chloride (7, 12, 17 mL) were added to a round bottom flask fitted with a condenser. The mixture was heated at a specific temperature (35, 60, 80ºC) in an oil bath. The mixture was mixed for a certain time (6, 13, 20 h) under magnetic stirring at an agitation speed of 500 rpm. The mixture was cooled down to room temperature and then, 10 mL of methanol were added to precipitate the carbohydrates. After the precipitation of carbohydrates, 10 mL of hexane were added in order to separate the lipids from the aqueous liquid phase. The aqueous liquid phase contains the water from primary sludge, the aqueous ionic liquid, methanol and part of the ashes and proteins. The organic phase containing hexane and lipids was separated by centrifugation for 10 min at 3500 rpm (Sorvall™ ST 16R Centrifuge). The operation was repeated 3 or 4 times. Then, the organic phase was dried over anhydrous sodium sulfate. Finally, hexane was evaporated in a rotary evaporator at 50ºC and 500 mbar for 10 min. The lipids were stored in a desiccator overnight and weighted the next day.

After separation of lipids, the precipitated solid was recovered from the aqueous liquid phase by centrifugation during 10 min at 6000 rpm. The recovered solid was washed with 10 mL of methanol. Then, the mixture was centrifuged (10 min, 6000 rpm) to separate solid and the supernatant. The washing operation was repeated 3 or 4 times (10 min, 6000 rpm). The remaining methanol of solid phase was evaporated in a rotary evaporator at 50ºC and 300 mbar for 20 min. The resulting solid was heated overnight at 105ºC and weighted.

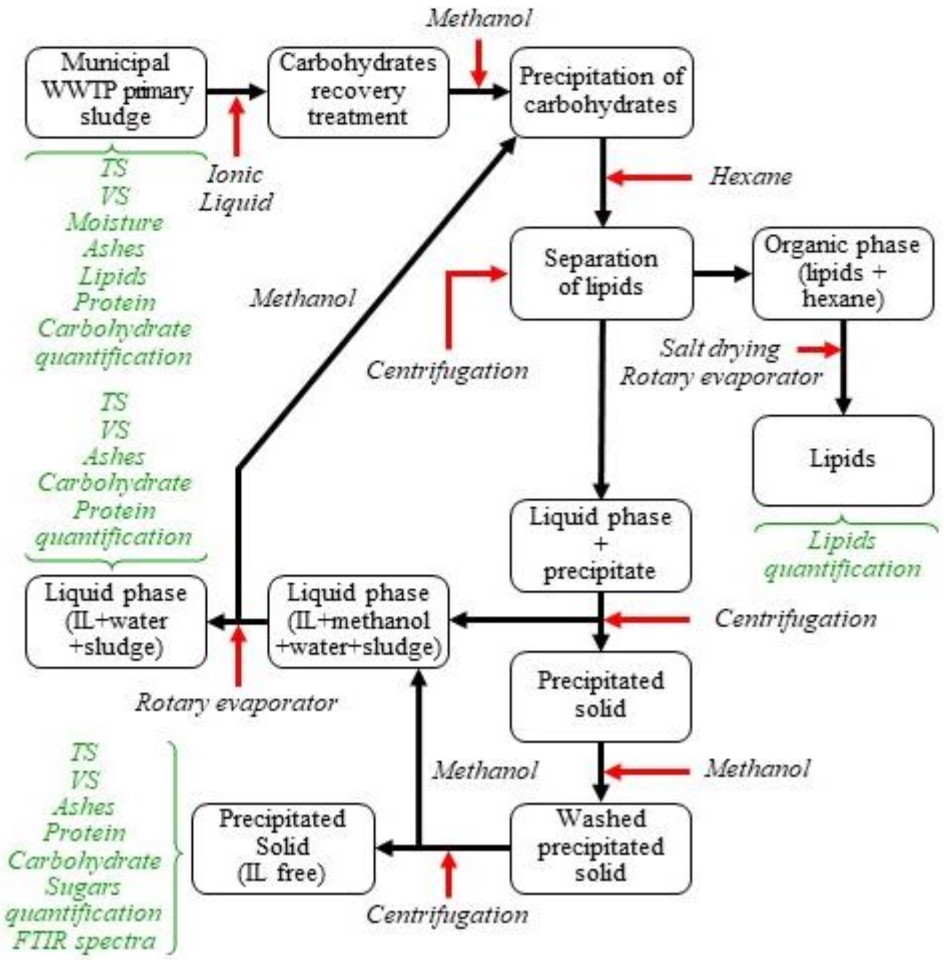

Figure 1. Recovery of cellulose from primary sludge using ionic liquid.

2.5. Characterization of the precipitated phase

The precipitate was analyzed in order to determine the volatile solids (VS) and ash content according to standard method 2540E (Rice et al., 2012). The weight of carbohydrates recovered was calculated as the difference between the weight of organic matter of precipitate and the weight of proteins in the precipitate. The amount of proteins in the precipitate was calculated as the difference of proteins in the primary sludge and the proteins determined in the aqueous liquid phase. The presence of cellulose in the precipitate was confirmed by FTIR spectroscopy.

Precipitate and standard cellulose samples, without any preparation, were scanned using a Fourier Jasco FT/IR-600 Plus spectrometer with a diamond golden gate ATR.

2.6. Characterization of the aqueous liquid phase

The presence of protein in IL phase was measured by a modified Lowry method at 500 nm (Olkiewicz et al., 2015a). Firstly, methanol was eliminated from aqueous liquid phase by evaporation at 50ºC and 300 mbar for 20 min. The absorbance of remaining liquid phase was measured at 500 nm due to have a better response than at 750 nm. The amount of ashes in aqueous liquid phase was calculated from the difference between the values of total ashes obtained in the wet primary sludge and the ashes analysed in the precipitate.

2.7. Characterization of carbohydrates in the precipitated solid

The content of cellulose in the precipitate was determined by a slightly modified National Renewable Energy Laboratory (NREL) method (Sluiter et al., 2008). Briefly, 100 mg of dry precipitate was dissolved in 2 mL of 72% sulfuric acid (instead of 300 mg in 3 mL of 72% H_2SO_4) and kept under agitation at 30ºC for 1 h. The acid solution was diluted to 2.5% with 56 mL of distilled water (in place of 84 mL), and transferred to an Erlenmeyer flask. The flask was placed in the autoclave at 120ºC and 1 bar for 1 h. The suspension was cooled down and filtered on a Whatman® glass microfiber filter (Grade GF/C 1.2 µm) previously weighted. The filtered solution was analyzed for sugars by HPLC with IR detector Agilent Technologies 1100 Series. An Agilent Hi-Plex H column conditioned at 60 ºC was used for all separations, using 5 mM sulfuric acid as mobile phase at a flow rate of 0.7 mL/min. The lignin content in solids was analyzed following a modified method of di Bitonto and co-workers (di Bitonto et al., 2016). The filtered solid was dried at 105 ºC for 24 h and then weighted. The lignin yield was calculated by the difference between the weight of the glass microfiber filter after drying and the weight of ashes obtained after burning the dried sample at 550 ºC for 1 h.

2.8. Optimization of process operative conditions through experimental design

The objective of using an experimental design was to optimize the recovery of cellulose from municipal wet primary sludge. For the optimization of the operative conditions of the process, a three level, three factor central design was applied (Dasari et al., 2017). A central composite design allows to predict the optimum reaction conditions for a maximum and/or minimum precipitated product yield. The influence on amounts of cellulose, hemicellulose, lignin, proteins and ashes depends on three independent factors: temperature of dissolution (x_1); time of dissolution (x_2); sludge: IL ratio (x_3) was investigated. Three levels for each factor were coded as -1; 0; 1. The experimental range and levels of independent factors are presented in Table 2. Values posed in the table were selected based on results of previous work (Olkiewicz et al., 2015a). During this study, 14 experiments have been carried out, 8 at the lower and higher values of the factors (-1; 1) and 6 at the central point of these factors (0). Table 3 presents the experimental conditions of the 14 experiments. For each substance, series of statistical hypothesis have been carried out to assess about a linear relation between the factors, at a particular level of each factor and at a global level. Also, for each substance again, it was assessed if there were interactions between the substance and the experimental values of each pair of factors. Finally, it was investigated if there was a quadratic behaviour in the values of component in the area of experimentation of the three factors.

Table 2. Experimental range and levels of independent variables.

Variables		**Range and levels**		
		Low level (-1)	**Center level (0)**	**High level (1)**
x_1	**Temperature (°C)**	35	60	85
x_2	**Time (h)**	6	13	20
x_3	**Ratio (g/TS:cm^3/IL)**	1:7	1:12	1:17

3. Results and discussion

3.1. Characterization of primary sludge

The results of characterization of the primary sludge are presented in Table 1. The wet primary sludge, as received, contains 96.1% of moisture. Total solids represent 3.9±0.1% of wet primary sludge. 85% of total solids are volatile solids, which is 3.3±0.2% of wet primary sludge in total solids basis. The rest, 0.6% in total solids basis, are ashes. Total solids contain 19.6 ± 0.6% of lipids (oil, greases, fats and long fatty acids); 27.7 ± 0.1% of proteins; 31.3 ± 0.1% of carbohydrates and 16.0 ± 0.1% of ashes. These results can be compared with those obtained by Olkiewicz (Olkiewicz et al., 2015a; Olkiewicz et al., 2015b), also showed in Table 1.

Table 3. Process conditions of experimental design.

Nº	**Temperature (ºC)**	**Time (h)**	**Ratio (g/TS:cm^3/IL)**
1	35	6	1:17
2	85	6	1:7
3	35	20	1:7
4	85	20	1:17
5	60	13	1:12
6	60	13	1:12
7	60	13	1:12
8	60	13	1:12
9	60	13	1:12
10	60	13	1:12
11	35	20	1:17
12	85	6	1:17
13	35	6	1:7
14	85	20	1:7

As it can be seen, the value of total solids is inferior by 7% to the value of 2015. This will be able to affect the quantity of carbohydrates and cellulose that will be recovered. On the contrary, the percentage of volatile sludge is equal in both works. The composition of the total solids, calculated in dry basis, is totally different. Values of lipids and ashes diminish and values of protein and carbohydrates increase. Differences are quite important, the higher is the decrease of lipids from 27.2% to 19.6% or the increase of carbohydrates from 26.2% to 31.3%. This last value is very interesting for the current study, focused in the recovery of cellulose. In three years, the sludge has changed considerably. These results, hardly predictable, are able to affect a future scaled process along time, increasing the difficulty to perform viability and economic feasibility evaluations.

3.2. Recovery of carbohydrates and other compounds from primary sludge with ionic liquid

3.2.1. Evaluation of the mixture during recovery of carbohydrates

The procedure of recovery of carbohydrates from municipal wet primary sludge was based on the know-how acquired during previous works (Olkiewicz et al., 2015a; Olkiewicz et al., 2015c). $[P(CH_2OH)_4]Cl$ ionic liquid was selected and used because of its high commercial availability and low cost over other ionic liquids (Fraser et al., 2009).

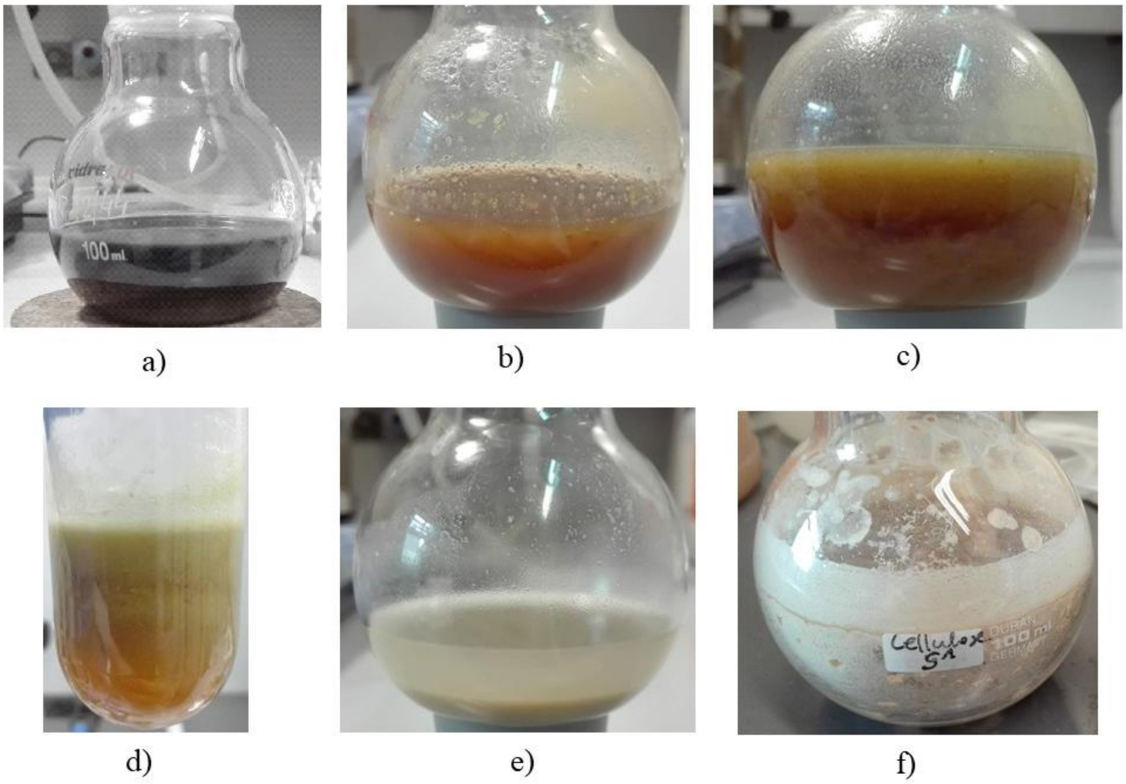

Figure 2. Pictures of the extraction stages using wet primary sludge and ionic liquid.

Figure 2 presents six pictures of the different consecutive steps of the procedure to recover carbohydrates from municipal wet primary sludge. Figure 2a shows the primary sludge with colorless ionic liquid before the reaction. Approximately 1 h after the start of the reaction, the solution changed color from black to dark orange (Figure 2b). After the dissolution of the carbohydrates, the addition of methanol caused the precipitation of the carbohydrates giving white fibrous material and an orange liquid phase (Figure 2c). The centrifugation of the mixture helped to separate fibrous material from supernatant (Figure 2d). Further methanol addition contributed to wash out the IL remained in the precipitate (Figure 2e). The remaining solvent of the solid was evaporated on a rotary evaporator giving whitish solid set on a flask wall (Figure 2f).

3.2.2. Recovery of lipids

Lipids are recovered after addition of hexane to the mixture. The organic phase containing hexane and lipids was separated by 3 or 4 times repeated centrifugation. Then, hexane was removed in a rotary evaporator. Finally, lipids were weighted. Table 4 shows the results of the quantity of lipids recovered for each experiment. It is possible to observe in the table that the quantity of lipids is more or less constant for all assays, except for the experiments 13 (85ºC; 20 h; 1:17), 10 (60ºC; 13 h; 1:12) and 13 (35ºC; 6 h; 1:7). It seems that the operative conditions do not affect very much the recovery of lipids from the sludge when extreme conditions are not used. The average value of lipids in these 11 experiments is 18.9 ± 0.6% w/w_{TS}. Experiment 4 presents the highest value of lipids, 21.4% w/w_{TS}, obtained with the most severe operative conditions. On the contrary, experiment 13 presents the lowest value of lipids, 15.2% w/w_{TS}, obtained with the less severe operative conditions. In this last case, the mass balance is not really right, and this can be a reason.

The average value is totally in accordance with the value of lipids obtained by the conventional Soxhlet method: 19.6 ± 0.6% w/w_{TS}. This signifies that the utilization of ionic liquids allows the recovery of the lipids from primary sludge, as it was suggested elsewhere (Olkiewicz et al., 2015a).

3.2.3. Proteins in the precipitate and in the aqueous phase

After separation of lipids, the precipitate was also separated from aqueous phase by centrifugation. The precipitate was then washed with methanol and centrifuged several times until total elimination of ionic liquid.

Table 4 also shows the values of the quantities of protein in the precipitate and in the aqueous liquid phase. The quantity of protein in the precipitate was calculated by a mass balance of proteins in the sludge and in the aqueous liquid phase. The precipitate still containing proteins. They were not totally removed from the polymer signifying that this ionic liquid is not totally able to remove proteins from carbohydrates. Another possibility is that these proteins were entrapped during precipitation of carbohydrates after the addition of methanol. This will be studied in the near future. The content of proteins remaining in the precipitate is lower than the total of proteins in the primary sludge, 53.6% of total proteins in average, but slightly higher than in the aqueous liquid phase, 46.4% of total proteins in average. Again, separation of protein from carbohydrates is not well achieved. On the other hand, values of protein in aqueous liquid phase for central experiments (5-10) are disappointing. Three values gave an average of 16.6% w/w_{TS} and the other three gave an average of 11.7% w/w_{TS}. This can be caused by an inhomogeneity of the composition of the primary sludge.

Table 4. Experimental results obtained after ionic liquid extraction (%).

Nº	**Organic phase**	**Precipitate**			**Aqueous-IL phase**		**Mass balance**
	Lipids	Proteins [a]	Carbohydrates [b]	Ashes	Proteins	Ashes [c]	
1	19,0	17,4	30,7	6,3	10,3	9,7	93,4
2	18,3	19,4	30,3	5,7	7,7	10,3	91,6
3	19,4	20,0	32,7	6,1	7,7	9,9	95,7
4	21,4	13,6	37,2	6,0	14,1	10,0	102,3
5	19,2	16,9	32,8	4,7	10,8	11,3	95,8
6	19,5	16,9	30,2	4,9	10,8	11,1	93,4
7	19,2	11,1	30,1	5,5	16,6	10,5	93,0
8	19,4	12,1	30,7	5,5	15,6	10,5	93,8
9	18,1	11,9	36,7	5,7	15,9	10,3	98,6
10	16,6	15,9	34,2	6,1	11,8	9,9	94,5
11	18,0	14,9	29,1	5,9	12,8	10,1	90,8
12	18,6	14,2	26,5	5,3	13,5	10,7	88,8
13	15,2	13,5	27,6	5,8	14,2	10,2	86,5
14	19,6	10,1	35,6	6,7	17,6	9,3	98,9

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

[b] Calculated from the difference between organic matter of the precipitate and the proteins in the precipitate.

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

3.2.4. Carbohydrates

The amount of carbohydrates in the precipitate was measured by the phenol-sulfuric acid Dubois method (Dubois et al., 1956). The values of the quantity of carbohydrates in the precipitate are correspondingly shown in Table 4. The average value of recovered carbohydrates is 31.7 ± 3.3%, based on total solids. This average value is significantly equal to the value obtained from dried primary sludge, also calculated by phenol-sulfuric acid Dubois method (Dubois et al., 1956), 31.3 ± 0.1%, based on total solids. This fact signifies that all carbohydrates are recovered in the precipitate. Moreover, if values out of the interval average ± standard deviation are removed from calculation, the new average value of recovered carbohydrates is 31.2 ± 1.6%, based on total solids. This value is then very close to the dried primary sludge value. This evidences that the utilization of ionic liquids is a good procedure to recover carbohydrates as it was planned. On the other hand, it seems that operative conditions do not have a big influence on the recovery of carbohydrates. The highest severe conditions, experiment 4 (85ºC; 20 h; 1:17), gave the highest value of carbohydrates, 37.2%, based on total solids, whereas the lowest severe conditions experiment 13 (35ºC; 6 h; 1:7), gave the lowest value of carbohydrates, 27.6%, based on total solids. However, these values should not be taken too much into consideration since in these experiments, mass balances are not good. In any case, it has been well demonstrated that the process with ionic liquids is capable of recovering the carbohydrates present in the primary sludge.

3.2.5. Ashes

The quantity of ashes in the precipitate was calculated by the conventional method 2540E (Rice et al., 2012). Table 4 also presents the values of ashes in the precipitate. The quantity of ashes in the precipitate is much lower than the amount calculated for the municipal primary sludge. The average value of ashes in the precipitate is 5.7 ± 0.5%, based on total solids. This value represents 35.8% of the containing of ashes in the municipal primary sludge. The reduction of ashes in the precipitate is notable, but not sufficient to avoid further cleaning of carbohydrates. The quantity of ashes in the aqueous liquid phase is calculated by the difference between values in municipal primary sludge and in the precipitate. The quantity of ashes in the aqueous liquid phase cannot be calculated by the conventional method 2540E (Rice et al., 2012) because the ionic liquid interferes in the procedure. More effective design of ionic liquids will be necessary to maintain the ashes in the liquid phase and not in the precipitate. Again, the operative conditions do not have a big influence on the removal of ashes from precipitate. During ionic liquid extraction beside carbohydrates and proteins, ashes also precipitate.

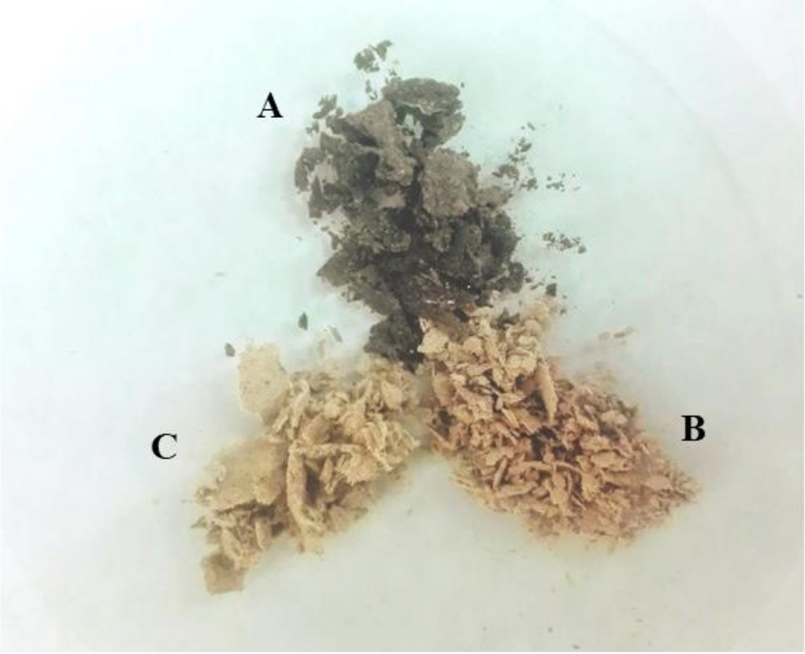

Figure 3. Precipitated material after different conditions, A: without IL, B: 1:7 sludge:IL ratio (g/TS:cm^3/IL) and C: 1:17 sludge:IL ratio (g/TS:cm^3/IL).

Figure 3 presents pictures of solids with and without utilisation of ionic liquids. Sample A, is dried municipal primary sludge. Its dark colour is caused by the high containing of ashes. Samples B and C are precipitates obtained after treatment with ionic liquids, ratios of 1:7 (B) and 1:17 (C). Part of proteins and ashes are removed. The lighter coloration of samples is caused by a lower quantity of ashes in the precipitate.

3.2.6. FTIR characterization of precipitate

In order to confirm the presence of cellulose in the carbohydrates, the precipitate was analyzed by FTIR. The spectrum was compared to a standard sample of pure cellulose. Figure 4 presents both spectra. It can be observed in the spectra the characteristic absorption bands of the carbohydrates. The broad peak around 1035 cm^{-1} presented in both spectra is assigned as a C-O stretching vibration of carbohydrates. A peak at 1160 cm^{-1} which is also presented in both spectra is assigned as the C-O-C stretching vibration. This peak is particularly associated with the presence of cellulose (Champagne and Li, 2009; Olkiewicz et al., 2015a). On the other hand, it is possible to observe characteristic bands at 1500-1700 cm^{-1} of peptide amide group of proteins. It means that during the separation, part of the proteins precipitated as well as it was confirmed by calculations.

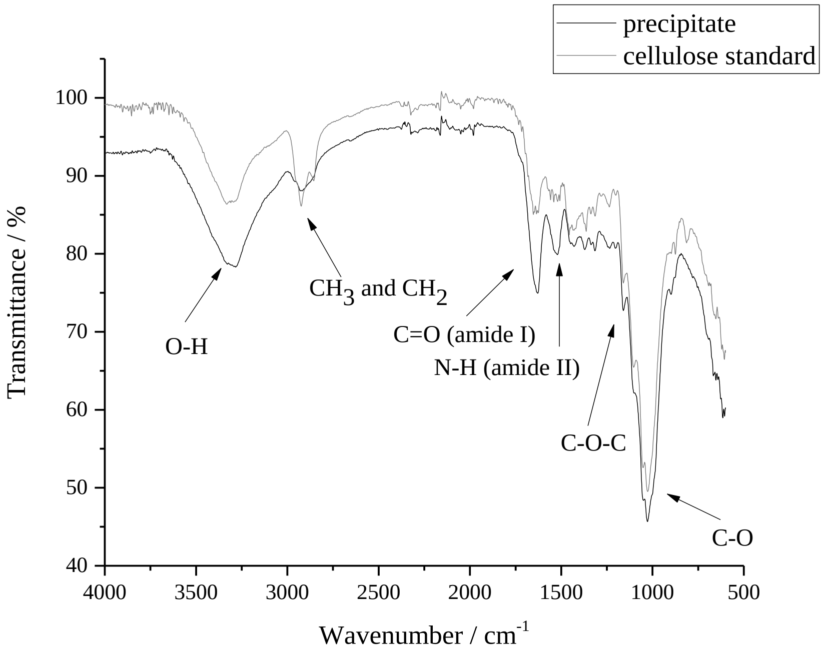

Figure 4. FTIR spectra of post reaction precipitate, dark line (experimental conditions: 60°C; 13 h; 1:12) and cellulose standard sample, grey line.

3.3. Statistical evaluation

The optimization of the recovery of cellulose was realized with a central factorial design. Three factors were used: temperature dissolution (x_1); time of dissolution (x_2) and ratio between weight of total solids and volume of ionic liquid (x_3). The values of the quantities of cellulose, hemicellulose, lignin, proteins and ash, all contained in the precipitate, were used to optimize the process and its factor of operation. The experimental results, allowed to carry out statistical hypothesis to assess whether there was a linear relation between the factors, at a particular level of each factor and at a global level. Moreover, for each substance of the precipitate, possible interactions between the substance and its experimental value were studied. This for each pair of factors and for a possible quadratic behavior with the three factors.

To do this, all experimental results were grouped in several categories: central experiments (5 to 10); temperature 35ºC (1, 3, 11 and 13); temperature 85ºC (2, 4, 12 and 14); time of dissolution 6 h (1, 2, 12 and 13); time of dissolution 20 h (3, 4, 11 and 14); ratio 1:7 (2, 3, 13 and 14); ratio 1:17 (1, 4, 11 and 12). Then, the average values for each substance was calculated for all categories. The results of these calculations are presented in Table 5. As it can be seen in the table, average values are quite constant for each substance independently of the categories. However, high standard deviations indicate that the values are spread out over a wider range. Therefore, it will be very difficult to obtain significant results at the statistical level, α=0.05, for the planned statistical tests. Seeing the values presented in the table, the six experiments with central points allow to recover high quantities of cellulose, to better remove quantities of ashes from precipitate, but also maintain high quantities of proteins in the precipitate.

Table 6 presents the p-values obtained from each of the statistical tests performed for cellulose, hemicellulose, lignin, proteins and ash. The conservative null hypothesis is that there are no differences in the results between all experiments performed at different conditions. The alternative hypothesis says that there are differences. To have differences, the p-value must be less than 0.05. As it can be seen in the table, in general, experimental conditions have few influences on the results, as linear, interactions and quadratic tests have p-values well over 0.05, except in limited cases.

More in detail, cellulose, as the most interesting substance, is not statistically influenced by any of the tests neither linear, interaction nor quadratic. All p-values are well over 0.05 except in the case of the interaction between temperature and time (p-value = 0.09).

In the case of hemicellulose, the interaction between temperature and sludge:IL ratio is statistically significant (p-value = 0.01). Moreover, the interactions between temperature and time and between time and sludge:IL ratio are also statistically significant (p-value = 0.03 for each). On the contrary, the test to assess the quadratic behaviour has a higher value (p-value = 0.13). Figure 5 presents the interaction between temperature and sludge:IL ratio for hemicellulose. As it can be seen in the figure, to obtain the high values of hemicellulose it is necessary to work using a low sludge:IL ratio and high temperature. The contrary, low temperature and high sludge:IL ratio also allows high quantities of hemicellulose.

Table 5. The average values of precipitated components obtained in different situation of temperature, time and sludge: IL ratio (g/100g precipitate, %).

Exp Nº	Temp (˚C)	Time (h)	Ratio	Cellulose	Hemicellulose	Lignin	Proteins	Ashes
5-10	60	13	1:12	25.6±5.7	8.6±0.9	28.4±4.2	26.0±3.2	10.4±1.2
1, 3, 11, 13	35			24.7±5.2	6.8±3.9	29.6±3.1	26.4±6.5	11.5±0.8
2, 4, 12, 14	85			25.8±5.0	8.6±0.9	29.6±3.6	23.6±4.2	11.3±1.1
1, 2, 12, 13		6		25.7±3.7	8.4±1.2	29.0±2.9	24.4±3.1	11.4±0.9
3, 4, 11, 14		20		24.8±6.3	7.0±3.9	30.2±3.6	25.6±7.4	11.4±1.1
2, 3, 13, 14			1:7	26.0±6.9	6.7±3.8	28.6±3.6	26.2±7.7	11.5±1.3
1, 4, 11, 12			1:17	24.5±2.2	8.7±1.0	30.7±2.5	23.8±1.1	11.4±0.6

Values are means ± SD, n=6 (exp 5-10) and n=4 (other series)

Table 6. p-values from the statistical tests performed for each precipitated component.

Test	Cellulose	Hemicellulose	Lignin	Proteins	Ashes
Lineal Temp.	0.78	0.24	0.99	0.42	0.79
Lineal Time	0.83	0.36	0.66	0.74	0.95
Lineal Ratio	0.70	0.19	0.46	0.48	0.93
Lineal Global	0.96	0.28	0.85	0.72	0.99
Inter Temp.-Time	0.09	0.03	0.24	0.04	0.45
Inter Temp.-Ratio	0.49	0.01	0.20	0.40	0.70
Inter Time-Ratio	0.96	0.03	0.78	0.78	0.77
Quadratic	0.92	0.13	0.59	0.68	0.23

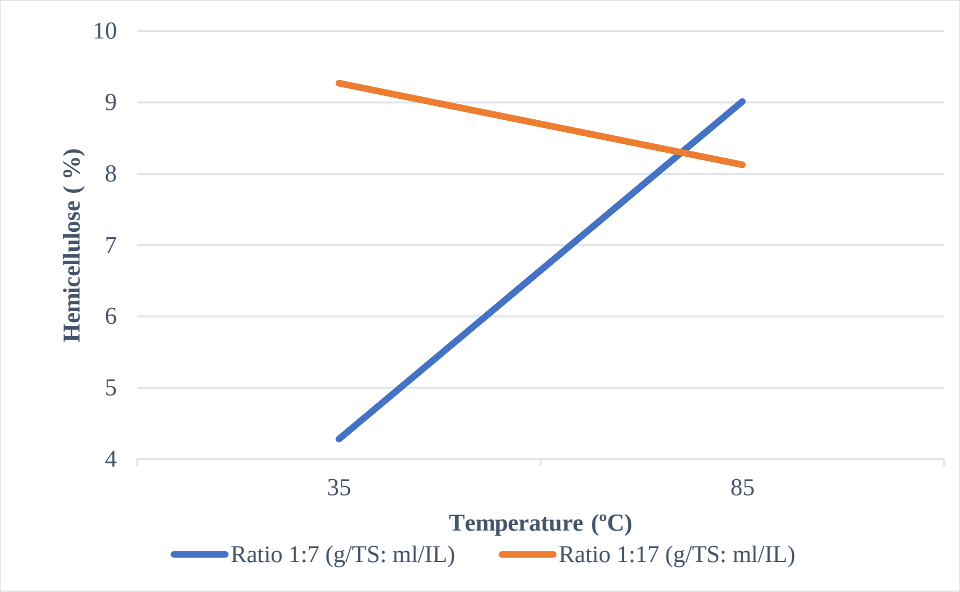

Figure 5. Interaction between temperature and sludge:IL ratio for hemicellulose.

In the case of the lignin, the behaviour is similar than for the cellulose, there is no statistically significant result, all p-values are well over 0.05.

For proteins, it is observed that the interaction between temperature – time is statistically significant (p-value = 0.04). For this substance, the highest values of proteins are obtained with the combination of low temperature and high time and with combination of high temperature and low time (Figure 6).

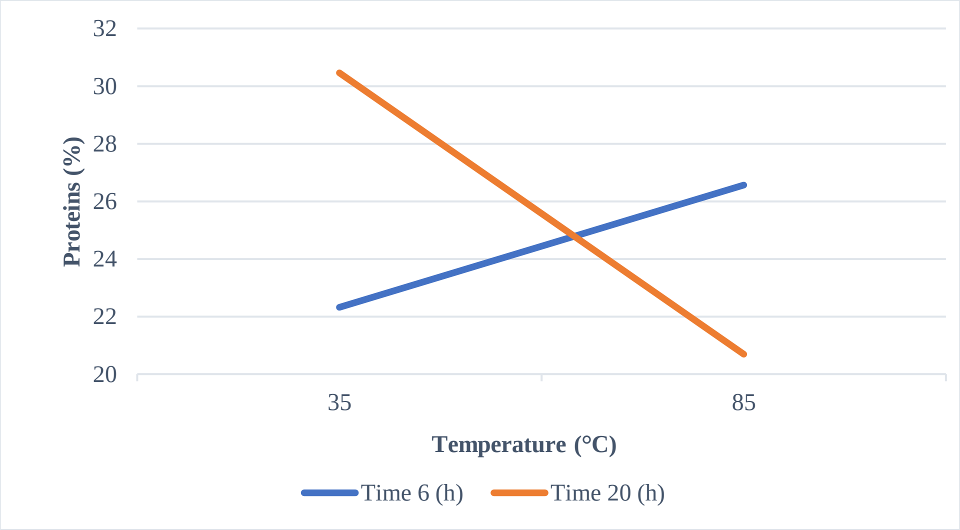

Figure 6. Interaction between temperature and time for proteins.

Finally, in the case of the ashes, again, there is no statistically significant result, all p-values are well over 0.05. The lowest, p-value = 0.23, corresponds to the test used to evaluate the quadratic behaviour.

3.4. Technical challenges of the study

The proposed technology presents great technological challenges with a great economic implication. First, the synthesis of ionic liquids is expensive. One of the most important challenges would be the production in large quantities of ionic liquids with an industrial cost. The relationship between primary sludge and ionic liquid is very unfavorable for this last one. Industrial scaling of the process would now require huge volumes of ionic liquid that are not currently possible. On the other hand, it should be noted that the profitability of the process can only be possible by treating large flows of primary sludge. The concentration of solids in this sludge is usually between 1 and 5%, the rest being essentially water. This implies that the ionic liquid is mixed with a large amount of water. In the case of the ionic liquid used in the process, Tetrakis (hydroxymethyl) phosphonium chloride (hydrated ionic liquid, 80% in water), assuming a ratio of 1 g of TS of wet primary sludge per 7 mL of ionic liquid, its concentration in water would fall up to 20%. Therefore, the second major challenge is the design of a continuous ionic liquid recovery process. The removal of much of the water to return to an ionic liquid concentration of 80% cannot be done by membrane separation processes. Works have shown that it is very difficult to concentrate the ionic liquid above 30% (Haerens et al., 2010). One possible solution is to concentrate the ionic liquid by the distillation of water. This is technically possible because the ionic liquid has a boiling point of 115 °C at atmospheric pressure which allows the distillation of the mixture. Continuous distillation processes of large flows are common in the chemical industry. On the other hand, the energy required to heat the mixture can be easily obtained. Indeed, if the process is installed in a WWTP, and it has an anaerobic digestion process of secondary sludge, then heat can be produced by the combustion of the produced biogas. After all, the redesign of WWTPs in true bio-refineries is only a question of time. As the ionic liquid has also effectively separated the lipids from the primary sludge, they can be used to produce biodiesel and make the cellulose recovery process more profitable (Olkiewicz et al., 2015a). The last big challenge is the design of improved ionic liquids able to solubilize the cellulose and remove all ashes and proteins. But this needs to be done by experts in the field (Plechkova and Seddon, 2008).

4. Conclusions

The optimization study about the recovery of cellulose from primary sludge was done. Precipitated solids were hydrolyzed and analyzed in order to quantify principal substances (cellulose, hemicellulose, lignin, proteins and ashes). The Tetrakis (hydroxymethyl) phosponium chloride ionic liquid has shown a great potential for the recovery of cellulose and others valuable components from the municipal WWTP primary sludge. The amount of carbohydrates measured by the Dubois method in the raw sludge and the average amount of carbohydrates recovered using the ionic liquid method is equal.

The ionic liquid allows the recovery of all cellulose, but it is not able to completely clean the precipitate of proteins and ashes. 36% of ashes and 51% of the proteins remain in the precipitate. This fact implies the need of a further cellulose bleaching before its valorisation. This should be avoided by the utilization of more selective ionic liquids, which implies a great improvement in their design.

The experimental design was not able to optimize the operative conditions of the process of recovery of carbohydrates. All conditions tested were able to recover maximal values of cellulose. From a statistical point of view, factors do not have an influence on the p-values except in few cases. Only interactions between temperature and sludge:IL ratio for hemicellulose and interaction between temperature and time for proteins were found.

Experiments realised with central values (60ºC; 13 h; 1:12) give very good results, allowing the recovery of high quantities of cellulose, the best removal of ashes but maintaining high quantities of proteins in the precipitate. For these reasons central values can be considered optimal reaction conditions.

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Chapter 5

Extraction of cellulose from corn stover using designed ionic liquids with improved reusing capabilities [1]

ABSTRACT

In recent years, the dissolution of lignocellulosic biomass using ionic liquids has attracted a lot of attention. Generally, the processes of valorisation of residual biomass do not extract cellulose. Among the lignocellulosic biomass, corn stover has a great interest because its high content in cellulose. For this reason, five ionic liquids have been designed and produced to dissolve the cellulose contained in corn stover, three tetra-alkyl-phosphonium based ILs and two imidazolium based ILs: tetra-butyl-phosphonium acetate $[P_{4444}][OAc]$; tri-butyl-methyl-phosphonium acetate $[P_{4441}][OAc]$; tetra-butyl-phosphonium 2-ethylhexanoate $[P_{4444}][EH]$; 1-dodecyl-3-methylimidazolium bis (2,4,4-tri-methyl-pentyl) phosphinate $[C_{12}mim][(^{i}C_8)_2PO_2]$; 1-decyl-3-methylimidazolium bis (2,4,4-tri-methyl-pentyl) phosphinate $[C_{10}mim][(^{i}C_8)_2PO_2]$. The dissolution of cellulose was carried out under mild conditions (3h and 80℃). Among the synthesised ionic liquids $[P_{4444}][EH]$ turned out to dissolve the greatest amount of cellulose (84%), while imidazolium ionic liquids $[C_{12}mim][(^{i}C_8)_2PO_2]$ and $[C_{10}mim][(^{i}C_8)_2PO_2]$ dissolved 61.1% and 44.0%, respectively. On the other hand, these designed ILs have the ability to be easily recovered. As they are microemulsion-forming ionic liquids, the recycling of these ionic liquids was more straightforward (without the need of solvent evaporation). Furthermore, imidazolium ionic liquids were found to eliminate more ashes from the corn stover than tetra-alkyl-phosphonium ionic liquids.

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[1] Glińska K., Giralt J., Torrens E., Plechkova N., Bengoa C. Extraction of cellulose from corn stover using designed ionic liquids with improved reusing capabilities. *Process Safety and Environmental Protection* 147C (2021).

1. Introduction

Corn stover contain stalks, leaves, husk and cobs that remain in the field after corn harvest. Its global annual production is almost equal to corn grain production and it is expressed as a ratio of dry matter of corn grain to residue as 1:1 (Sun et al., 2019). According to literature (Ruan et al., 2019), the global corn stover production is around 1 billion tons. Corn stover is mainly used as feed for animals and livestock bedding. Also, some part of it is left in the field to maintain soil productivity (Ruan et al., 2019). Corn stover is an abundant resource of renewable feedstock due to its composition. It is composed of three main polymers which are cellulose (~35% w/w), hemicellulose (~20% w/w) and lignin (~12% w/w). Cellulose properties such as: biocompatibility, biodegradability, thermal and chemical stability (Mohd et al., 2017) allow it to be processed into value added molecules, building bio-blocks, bioplastics and flocculants (De Clercq et al., 2017; Salehizadeh et al., 2018; Badgujar et al., 2019). In order to release cellulose fibers, which are embedded in a solid matrix of lignin and hemicelluloses (Singhet al., 2018), pretreatment processes of biomass must be performed. These processes can be divided in four main groups: physical, chemical, physicochemical and biological (Baruah et al., 2018). In this study, chemical pretreatment processes using ionic liquids (ILs) have been studied.

ILs are an environmentally friendly alternative to classic organic solvents that are volatile and harmful (Seddon, 1997; Plechkova and Seddon, 2008). ILs are salts with melting point temperatures below 100℃. They are composed of an organic bulky cation and an organic or inorganic anion, that it can also be bulky (Wahlstrom and Suurnakki, 2015). Generally, ILs have an excellent chemical and thermal stability. Biomass processing using ionic liquids began in 2002 (Swatloski et al., 2002). Since then, biomass processing using ILs has become used increasingly (Huang et al., 2008; Li et al., 2009; Tadesse and Luque, 2011; Froschauer et al., 2013; Smiglak et al., 2014; Khare et al., 2015; Loow et al., 2017; Ullah et al., 2019).

One of the characteristics of these ionic liquids is their ability to break the cell walls of lignocellulosic biomass, allowing the dissolution of the cellulose. Swatlowski and co-workers (Swatloski et al., 2002), described how imidazolium ionic liquids are able to dissolve cellulose and among these 1-butyl-3-methyl-imidazolium chloride [C_4mim]Cl produced the best results. 10% of the cellulose was dissolved when mixture was heated to 100°C. Microwave heating allowed to dissolve 25% of the cellulose. After the dissolution, cellulose is precipitated by adding a polar solvent to the mixture (Olkiewicz et al., 2015). Then, the recovery of the ionic liquid is only possible after evaporation/distillation of the solvent which results in high energy consumption (Kuzmina and Hallett, 2016). One of the disadvantages of ionic liquids is their perceived high price, although some of them, especially those with non-halogenated anions can be manufactured inexpensively (Brandt et al., 2013).

The ILs able to be easily recovered are the ILs which would be immiscible with a polar solvent responsible of the precipitation of the cellulose. Between them, tetra-butyl-phosphonium acetate $[P_{4444}][OAc]$ has a well-known ability to dissolve cellulose (Abe et al., 2015). Its short alkyl chain carboxylate anion, which is polar and has a strong H-bond-accepting ability (Otero et al., 2017), allows this IL to mix with a polar solvent. In turn, tri-butyl-methyl-phosphonium acetate $[P_{4441}][OAc]$ and tetra-butyl-phosphonium 2-ethyl-hexanoate $[P_{4444}][EH]$ must show their ability to dissolve cellulose and be miscible with solvent. Their configuration with a symmetrical cation or not and a longer chain anion must have influence on the dissolution and on their miscibility (Abe et al., 2015).

To make the separation of cellulose possible without the need of evaporating a polar solvent from the precipitated cellulose (which is energy-consuming), the concept of microemulsions can be used (Hejazifar et al., 2016). In the same way, the microemulsion-forming imidazolium ionic liquids, 1-dodecyl-3-methyl-imidazolium bis-(2,4,4-tri-methyl-pentyl)-phosphinate $[C_{12}mim][(^iC_8)_2PO_2]$ and 1-decyl-3-methyl-imidazolium bis-(2,4,4-tri-methyl-pentyl)-phosphinate $[C_{10}mim][(^iC_8)_2PO_2]$, were prepared (Collin et al., 2006). Except of the microemulsion-formation concept, imidazolium-based ILs were chosen for their known ability to dissolve cellulose (Uto et al., 2018). To the best of our knowledge, neither of the aforementioned ionic liquids have ever been used for cellulose dissolution from lignocellulosic biomass.

The aim of this work is to synthesize and identify ILs capable of breaking lignocellulosic biomass walls with the added ability of cellulose dissolution. Furthermore, these ionic liquids could be easily recovered after reaction. Corn stover was used as a lignocellulosic material.

2. Materials and methods

2.1. Reagents

Tetra-butyl-phosphonium hydroxide, $[P_{4444}][OH]$, ≈ 40 wt% in water was purchased from TCI Chemicals. Ethanoic acid (≥99%), 2-ethyl-hexanoic acid and sodium hydroxide were purchased from Sigma-Aldrich. CYPHOS IL 340 (tri-butyl-methyl-phosphonium methyl carbonate) (74%) was kindly donated by Solvay. 1-decyl-3-methyl-imidazolium chloride $[C_{10}mim]Cl$ and 1-dodecyl-3-methyl-imidazolium chloride $[C_{12}mim]Cl$ were already synthesised according to the procedure of Hejazifar and co-workers (Hejazifar et al., 2016). Bis (2,4,4-tri-methyl-pentyl) phosphinic acid, commonly known as a Cyanex 272, was supplied by Solvay. Ethanol 95%, methanol, toluene, hexane and 96% sulfuric acid were provided by Sigma-Aldrich. All solvents and reagents were high performance chromatography grade and analytical reagent grade.

2.2. Synthesis and characterization of ILs

Below are presented the procedures of synthesis of the five ILs. The ionic liquids were characterised by ^{1}H NMR and ^{13}C NMR (Bruker Avance III spectrometer) spectroscopy.

IL1: 1-Dodecyl-3-methylimidazolium bis-(2,4,4-tri-methyl-pentyl)-phosphinate, $[C_{12}mim][(^{i}C_8)_2PO_2]$

$[C_{12}mim][(^{i}C_8)_2PO_2]$ was exactly synthesised according to the method described in literature (Hejazifar et al., 2016).

^{1}H NMR (d^4-methanol, 400 MHz); δ (ppm): 0.51-0.59 (m, 21H); 0.74-0.78 (m, 6H); 0.92-1.04 (m, 18H); 1.22 (m, 2H); 1.53 (m, 2H); 1.66 (m, 2H); 2.96 (s, 2H); 3.60 (s, 3H); 3.89 (t, 2H, J = 7.2 Hz); 7.30 (t, 1H); 7.35 (t, 1H); 8.93 (s, 1H).

^{13}C NMR (d^4-methanol); δ (ppm): 14.85 (CH_3); 23.78 (CH_2); 25.15 (2 × CH_3); 26.50 (2 × CH); 27.33 (CH_2); 30.24 (CH_2); 30.78 (5 × CH_2); 31.04 (2 × C); 31.32 (6 × CH_3); 32.17 (CH_2); 33.08 (CH_2); 36.62 (CH_3); 42.84 (P-CH_2); 43.72 (P-CH_2); 49.89 (CH_2); 54.93 (2 × CH_2); 123.58 (CH); 125.00 (CH); 138.23 (CH).

IL2: 1-Decyl-3-methylimidazolium bis-(2,4,4-tri-methyl-pentyl)-phosphinate, $[C_{10}mim][(^{i}C_8)_2PO_2]$

$[C_{10}mim][(^{i}C_8)_2PO_2]$ was exactly synthesised analogous to $[C_{12}mim][(^{i}C_8)_2PO_2]$, following the same work (Hejazifar et al., 2016).

^{1}H NMR (d^4-methanol, 400 MHz); δ (ppm): 0.86-0.95 (m, 21H); 1.09-1.11 (m, 6H); 1.28-1.44 (m, 18H); 1.63 (m, 2H); 1.88 (m, 2H); 1.96 (m, 2H); 3.95 (s, 3H); 4.24 (t, 2H, J = 7.6 Hz); 7.58 (s, 1H); 7.60 (s, 1H); 8.98 (s, 1H).

^{13}C NMR (d^4-methanol); δ (ppm): 14.74 (CH_3); 23.52 (CH_2); 24.74 (2 × CH_3); 26.25 (2 × CH); 27.12 (CH_2); 30.03 (CH_2); 30.21 (4 × CH_2); 30.48 (2 × C); 30.92 (6 × CH_3); 31.91 (CH_2); 32.81 (CH_2); 36.72 (CH_3); 41.72 (P-CH_2); 42.59 (P-CH_2); 54.62 (2 × CH_2); 123.35 (CH); 124.89

(CH); 137.24 (CH).

IL3: Tetra-butyl-phosphonium acetate, $[P_{4444}][CH_3COO]$

Ethanoic acid (9.569 g, 0.159 mol) was added dropwise to the aqueous $[P_{4444}][OH]$ (110.129 g, 0.159 mol) in equimolar quantities. The mixture was stirred overnight at room temperature and atmospheric pressure before being transferred to a rotary evaporator (4 h, 40ºC) to remove the bulk water. The $[P_{4444}][CH_3COO]$ was stirred, heated *in vacuo* (80ºC, 48 h, *ca.* 0.01 mbar) and obtained as a colourless liquid (yield 97%).

^{1}H NMR (D_2O, 400 MHz, 353.15 K); δ (ppm): 0.94 (t, 12H, *J* = 8 Hz); 1.40-1.63 (m, 16H); 1.92 (s, 3H); 2.10-2.24 (m, 8H).

^{13}C NMR (D_2O); δ (ppm): 12.93 ($4 \times CH_3$); 17.45 ($4 \times CH_2$); 17.93 ($4 \times CH_2$); 22.86-23.46 ($4 \times P\text{-}CH_2$); 23.85 (CH_3); 179.96 (C=O).

IL4: Tri-butyl-methyl-phosphonium acetate, $[P_{4441}][CH_3COO]$

Ethanoic acid (16.194 g, 0.269 mol) was added dropwise to the aqueous tri-butyl-methyl-phosphonium methyl carbonate $[P_{4441}][MeCO_3]$ (104.032 g, 0.269 mol) in equimolar quantities. The mixture was stirred overnight at room temperature and atmospheric pressure before being transferred to a rotary evaporator (3 h, 40ºC) to remove the bulk water, methanol and carbon dioxide. The $[P_{4441}][CH_3COO]$ was stirred, heated *in vacuo* (80ºC, 48 h, *ca.* 0.01 mbar) and obtained as a light yellow liquid (yield 95%).

^{1}H NMR (D_2O, 400 MHz, 353.15 K); δ (ppm): 0.93 (t, 9H, *J* = 4 Hz); 1.40-1.63 (m, 12H); 1.77 (d, 3H, *J* = 8Hz); 1.93 (s, 3H); 2.10-2.23 (m, 6H).

^{13}C NMR (D_2O); δ (ppm): 12.90 ($3 \times CH_3$); 18.99 ($3 \times CH_2$); 19.48 ($3 \times CH_2$); 22.80-23.40 ($3 \times P\text{-}CH_2$); 23.63 ($P\text{-}CH_3$); 31.36 (CH_3); 179.37 (C=O).

IL5: Tetra-butyl-phosphonium 2-ethylhexanoate, $[P_{4444}][CH_3(CH_2)_3CH(C_2H_5)COO]$

Ethyl-hexanoic acid (21.517 g, 0.149 mol) was added dropwise to the aqueous $[P_{4444}][OH]$ (103.118 g, 0.149 mol) in equimolar quantities. The mixture was stirred overnight at room temperature and atmospheric pressure before being transferred to a rotary evaporator (3 h, 40ºC) to remove the bulk water. The $[P_{4444}][CH_3(CH_2)_3CH(C_2H_5)COO]$ was stirred, heated *in vacuo* (80ºC, 48 h, *ca.* 0.01 mbar) and obtained as a colourless liquid (yield 98%).

^{1}H NMR (D_2O, 400 MHz, 353.15 K); δ (ppm): 0.81-0.88 (m, 6H); 0.91 (t, 12H, $J = 8$ Hz); 1.19-1.35 (m, 4H); 1.19-1.35 (20H); 2.06-2.24 (m, 9H).

^{13}C NMR (D_2O); δ (ppm): 12.06 (CH_3); 12.95 ($4 \times CH_3$); 13.86 (CH_2); 17.59 ($4 \times CH_2$); 18.07 ($4 \times CH_2$); 22.75 (CH_2); 23.00-23.52 ($4 \times$ P-CH_2); 26.04 (CH_2); 29.89 (CH_3); 32.60 (CH_2); 50.89 (CH); 184.16 (C=O).

2.3. Water miscibility properties of ILs

Distilled water and IL (50/50 wt%) were mixed in a vial and stirred. Then, the mixture was cooled or heated to the fixed temperature 0-70℃ and the miscibility was confirmed by the presence after decantation of one or two phases.

2.4. Corn stover characterization

The stover was ground with a Taurus Aromatic coffee grinder to pass through a 0.2 mm sieve. Moisture (%M) of the stover was calculated by the difference between the mass sample before and after drying at 105℃ for 24 h. The extraction of waxes, fats and resins was performed according to literature (Moore and Johnson, 1967), changing the benzene for toluene. Ash content was measured following the same protocol (Moore and Johnson, 1967). A slightly modified method to the one described in literature (Sambusiti et al., 2016) was used to determine total soluble sugars, cellulose, hemicellulose and lignin content in the corn stover. 100 mg of dry corn stover was hydrolyzed in 5 mL of distilled water at 100℃ for 1h. Then, the supernatant was filtered through a syringe filter (0.22 µm) and analysed by HPLC, as described below. Carbohydrates (cellulose, hemicellulose) and lignin were measured using strong sulfuric acid hydrolysis. 100 mg of the dry stover was dissolved in 1 ml of 72% sulfuric acid and kept under agitation for 1 h at room temperature. Then, the acid solution was diluted with 17 mL of distilled water to 4% and autoclaved at 121℃ for 1 h. The suspension was cooled to room temperature and filtered on a Whatman® glass microfiber filter (Grade GF/C 1.2 µm) previously weighted. The filtered solids were abundantly washed with distilled water. Lignin was determined by drying the filtered solid at 105°C for 24 h and then weighting it. The lignin yield was calculated by the difference between the weight of the glass microfiber filter after drying and the weight of ashes obtained after burning the dried sample at 550°C for 3 h. The filtered solution was analyzed for carbohydrates by HPLC with IR detector Agilent Technologies 1100 Series. An Agilent Hi–Plex H column, conditioned at 60°C, was used for all separations using 5 mM sulfuric acid from the mobile phase at a flow rate of 0.7 mL/min. The sample of corn stover, without any further preparation, was scanned by an electronic scanning microscope, (SEM) JEOL model JSM-6400, in order to carry out the elemental microanalysis of the biomass. For an accurate analysis, the corn stover was scanned in different areas of the sample.

2.5. Cellulose extraction by imidazolium ILs

Figure 1 represents the scheme of the cellulose extraction from the corn stover by imidazolium ionic liquids following the procedure of Olkiewicz and co-workers (Olkiewicz et al., 2015). In a round bottomed flask fitted with condenser dry, extractive–free corn stover (0,5 g) was mixed with IL (5 g). Then, the mixture was stirred for 3 h at 80℃. Afterwards, the mixture was cooled to room temperature and water (20 mL) was added to precipitate the solubilised cellulose, followed by hexane (38.2 mL) in order to form a microemulsion (Hejazifar et al., 2016). Post reaction corn stover, with extracted cellulose/water/IL/hexane solution, was centrifuged (4400 rpm, 15 min) in order to improve the phase separation. Each phase was gathered separately and their composition tested by ^{1}H NMR spectroscopy. A DMSO-d^6 capillary tube was used as an internal reference. Post reaction corn stover was washed with methanol to remove traces of the IL, dried overnight at 105℃ and weighed. After that, the presence of cellulose in the treated corn stover was confirmed by FTIR spectroscopy. The samples of initial and post reaction corn stover, without any preparation, were scanned by a Fourier Jasco FT/IR–600 Plus spectrometer with a diamond golden gate ATR. The collection of the single beam background and sample spectra was realised with 32 scans at 4 cm^{-1} resolution.

2.6. Cellulose extraction by phosphonium ILs

In the case of phosphonium ILs (Figure 1), the procedure for cellulose extraction was the same as above until the heating stage. Then, the mixture was cooled to room temperature. Due to the fact that the selected phosphonium ILs are not able to form microemulsions, only methanol (10 mL) was added. After cellulose precipitation, the mixture was poured to the centrifuge tube and centrifuged (6000 rpm, 10 min) to separate the post reaction corn stover from the extracted cellulose and IL/methanol solution. Afterwards, the solid was washed with methanol (10 mL)

3-4 times in order to remove any traces of IL. Free IL post reaction corn stover was dried overnight at 105℃ and weighed. As in the previous procedure, the presence of cellulose in the post reaction corn stover was confirmed by FTIR spectroscopy.

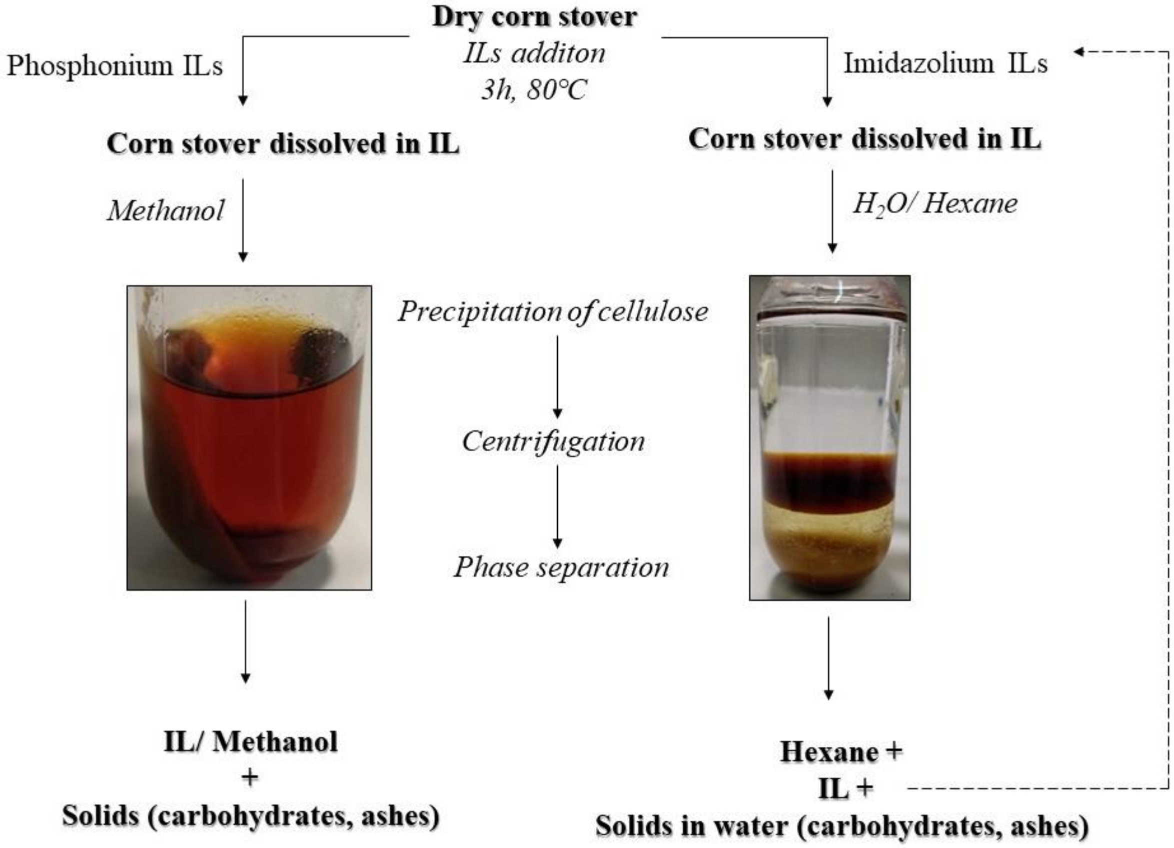


Figure 1. Scheme of the experimental procedure of extraction of cellulose from corn stover with phosphonium and imidazolium ionic liquids.

2.7. Post reaction corn stover characterization

After each reaction, ash content in the post reaction corn stover was determined according to literature (Moore and Johnson, 1967). The amount of extracted cellulose was measured based on a quantitative analysis of the powder solids with FTIR-ATR Jasco IR application note 02-03. A number of standards of stover plus commercial cellulose mixtures of known concentration (0-6 mg) were prepared. Fresh corn stover was considered as having a standard 0 mg of extracted cellulose. The absorbance of the bands associated with the cellulose (1035 cm^{-1} and 1160 cm^{-1}) from aliquots of the standards was collected. 32 scans at 4 cm^{-1} resolution were used to collect the single beam background and sample spectra. Then, least square plot of the ATR data was performed. After each reaction, sample aliquots of 10 mg were placed onto an ATR crystal and spectra were collected without additional sample preparation. The amount of extracted cellulose was calculated by interpretation of the tendency equation obtained from the plot made with the samples. The absorbance used for the calculation of extracted cellulose after reaction was the difference between the absorbance of a sample and the absorbance of the standard 0.

2.8. Reuse of imidazolium ILs

After the phase separation, the ionic liquid solution was transferred to a round bottom flask in order to evaporate the water and hexane residuals. Then the structure of the recovered ionic liquid was checked by ^{1}H NMR spectroscopy. Cellulose dissolution from the corn stover was also carried out again according to the method described above using the imidazolium ILs as recovered, without water nor hexane evaporation. This process was performed in order to check if the recovered imidazolium ILs were still able to dissolve cellulose omitting energy-consuming step of evaporation of the water and hexane residuals.

3. Results and discussion

3.1. Characterization of corn stover

The results of the characterization of the corn stover are presented in Table 1. Values were calculated based on 100 g of dry corn stover. As it can be seen in the table, the corn stover consists of waxes, fats and resins; ash; hemicellulose (xylose, arabinose), cellulose (built by glucose), lignin and a small amount of sugars soluble in water. Glucose is the main component of the biomass. Based on glucose, the amount of cellulose can be calculated from equation (1) (Sambusiti et al., 2016):

$$\text{Cellulose (\%/TS)} = \text{Glucose (\%)} / 1.11 \qquad (1)$$

where 1.11 is the conversion factor for glucose to monomers (Sambusiti et al., 2016).

The experimental value of glucose obtained for corn stover was 39.1 ± 0.5%, w/w_{TS} (Table 1). Applying to this value the conversion factor used to determine the quantity of cellulose, 1.11, this results in a value of cellulose of 35.2 ± 0.5%, w/w_{TS}. This value of cellulose will be used for further calculation operations in section 3.6 (Table 3). The results for polymers are comparable to literature (Wan and Li, 2010). An elemental microanalysis presented peaks for carbon and oxygen corresponding to carbohydrates, silica and potassium corresponding to ashes. On the other hand, the presence of nitrogen in the sample was not observed, thus the amount of proteins is little. According to the literature (Guo et al., 2020), corn stover contain around 5% of proteins.

Table 1. Characterization of corn stover and post reaction corn stover by conventional procedures.

	Corn stover	**IL1**	**IL2**	**IL3**	**IL4**	**IL5**	**Reused IL1**	**Reused IL2**
Moisture (%, w/w_{wetCS})	7.3 ± 0.1	11.0 ± 4.6	12.8 ± 3.1	9.1 ± 1.5	7.8 ± 1.6	6.5 ± 2.3	16.2 ± 1.1	17.0 ± 1.3
TS (%, w/w_{wetCS})	92.7 ± 0.1	89.0 ± 4.6	87.2 ± 3.1	90.9 ± 1.5	92.2 ± 1.6	93.5 ± 2.3	83.8 ± 1.1	83.0 ± 1.3
Lipids/Extractives (%, w/w_{TS})	3.5 ± 0.9	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.
Ash (%, w/w_{TS})	5.1 ± 0.0	3.4 ± 0.0	2.7 ± 0.0	4.3 ± 0.9	4.3 ± 0.9	4.1 ± 0.0	3.0 ± 0.0	2.3 ± 0.8
Xylose (%, w/w_{TS})	21.6 ± 0.3	18.7 ± 0.6	18.8 ± 0.2	19.7 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	16.8 ± 0.1	16.9 ± 0.1
Arabinose (%, w/w_{TS})	4.6 ± 0.1	3.7 ± 0.0	3.6 ± 0.1	3.8 ± 0.1	3.8 ± 0.0	3.8 ± 0.1	2.9 ± 0.2	3.0 ± 0.2
Glucose (%, w/w_{TS})	39.1 ± 0.5	34.0 ± 1.3	35.7 ± 0.4	34.6 ± 0.1	35.7 ± 0.1	35.9 ± 0.2	32.4 ± 0.0	32.3 ± 0.1
Lignin (%, w/w_{TS})	16.9 ± 0.4	13.2 ± 2.1	12.0 ± 0.3	13.5 ± 0.5	11.3 ± 1.4	9.1 ± 3.9	13.3 ± 0.4	13.2 ± 0.8
Sugars soluble in water (%, w/w_{TS})	1.2 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	N.R.	N.R.	N.R.	0.3 ± 0.0	0.9 ± 0.0
Total * (%, w/w_{TS})	92.0 ± 2.2	73.1 ± 4.0	72.9 ± 1.0	75.9 ± 1.7	74.3 ± 2.5	72.1 ± 4.3	68.7 ± 0.7	68.6 ± 2.0

IL1: $[C_{12}mim][(^{i}C_8)_2PO_2]$
IL2: $[C_{10}mim][(^{i}C_8)_2PO_2]$
IL3: $[P_{4444}][CH_3COO]$
IL4: $[P_{4441}][CH_3COO]$
IL5: $[P_{4444}][CH_3(CH_2)_3CH(C_2H_5)COO]$
Values are means ± SD, n = 3
TS: Total Solids
*Total: Lipids + Ash + Xylose+ Arabinose +Glucose + Lignin +Sugars soluble in water

3.2. Properties of synthesised ionic liquids

The properties of each synthesised IL are presented in Table 2. Tetra-alkyl-phosphonium cations, with a similar number of carbon atoms in total, were compared in terms of miscibility with a polar solvent (water) and in the terms of cellulose dissolution. As it is known (Abe et al., 2015), the hydrophilic property depends on the length of the alkyl chain; hydrophily generally decrease with increasing alkyl chain length. In this case, all tetra-alkyl-phosphonium ionic liquids showed the ability to be totally miscible with water (hydrophilic). Tri-butyl-methyl-phosphonium acetate was the ionic liquid with the smallest number of carbon atoms (15) and as expected, was totally miscible with water. Tetra-butyl-phosphonium 2-ethyl-hexonate has the largest alkyl chain among the other phosphonium based ILs synthetized during the work. It was assumed, that a longer alkyl chain would have an influence on the miscibility of the IL with water. However, the miscibility of Tetra-butyl-phosphonium 2-ethyl-hexonate with water is also 100%, totally miscible, like for Tri-butyl-methyl-phosphonium acetate IL. The goal of making a phosphonium-based ionic liquid immiscible with water has not been achieved.

Table 2. Water miscibility and cellulose dissolution test of synthesized ILs.

Ionic liquid	Water miscibility with ionic liquid	Cellulose solubility in ionic liquid at 80℃
IL1: $[C_{12}mim][({}^{i}C_8)_2PO_2]$	Microemulsion *	Soluble
IL2: $[C_{10}mim][({}^{i}C_8)_2PO_2]$	Microemulsion *	Soluble
IL3: $[P_{4444}][CH_3COO]$	Miscible	Soluble
IL4: $[P_{4441}][CH_3COO]$	Miscible	Soluble
IL5: $[P_{4444}][CH_3(CH_2)_3CH(C_2H_5)COO]$	Miscible	Soluble

* Ability to bring hydrophobic and hydrophilic (hexane/water) solvents together as stable microemulsions (Hejazifar et al., 2016).

The structure of the ionic liquid as well as its properties, have an influence on the solubilization of the cellulose. Cellulose can be dissolved in ILs containing anions that have the ability to form hydrogen bonds with the hydroxyl groups present in cellulose, for example, chloride, acetate, or amino acids (Capolupo and Faraco, 2016). As all tetra-alkyl-phosphonium ionic liquids had anions that permitted to form hydrogen bonds, they were able to dissolve the cellulose of the corn stover. The effect of cation on cellulose dissolution was also verified (Li et al., 2018). It was observed that IL with a symmetrical as an unsymmetrical cation was able to dissolve the cellulose. As a conclusion, the ionic liquids with these both cations can be used to dissolve cellulose. However, they cannot be called easily recoverable ILs because they are miscible with polar solvent.

Two imidazolium ionic liquids are also presented In Table 2. Both ILs differ by the number of carbon atoms in the cation chain. Those ILs are known as surface-active ionic liquids (Hejazifar et al., 2016). They are not miscible with water, but they have the ability to form stable microemulsions. Since the solubility of cellulose is highest when ILs are based on imidazolium, pyridinium and ammonium cations (Gupta and Jiang, 2015), they were able to dissolve the cellulose of corn stover.

In order to confirm the presence of cellulose post reaction, FTIR spectra of the five treated with ILs corn stover were recorded (Figure 2). The post reaction spectra (Figure 2b for IL1, Figure 2c for IL2, Figure 2d for IL3, Figure 2e for IL4 and Figure 2f for IL5) were compared with the FTIR of the initial corn stover (Figure 2a). All spectra showed some characteristic peaks: 3400 cm^{-1} for the hydroxyl group and 2950 cm^{-1} and 1000-1250 cm^{-1} attributed to -CH and -C-O of carbohydrates. However, differences between the spectra were observed. In post reaction spectra (Figures 2b to 2f), corn stover showed a peak around 1159 cm^{-1} which was not observed in the initial corn stover sample (Figure 2a). That peak is assigned to the C–O–C stretching vibration and it is particularly associated with cellulose (Champagne and Li, 2009; Olkiewicz et al., 2015). This confirms that after the reaction with the ILs, cellulose was released from corn stover.

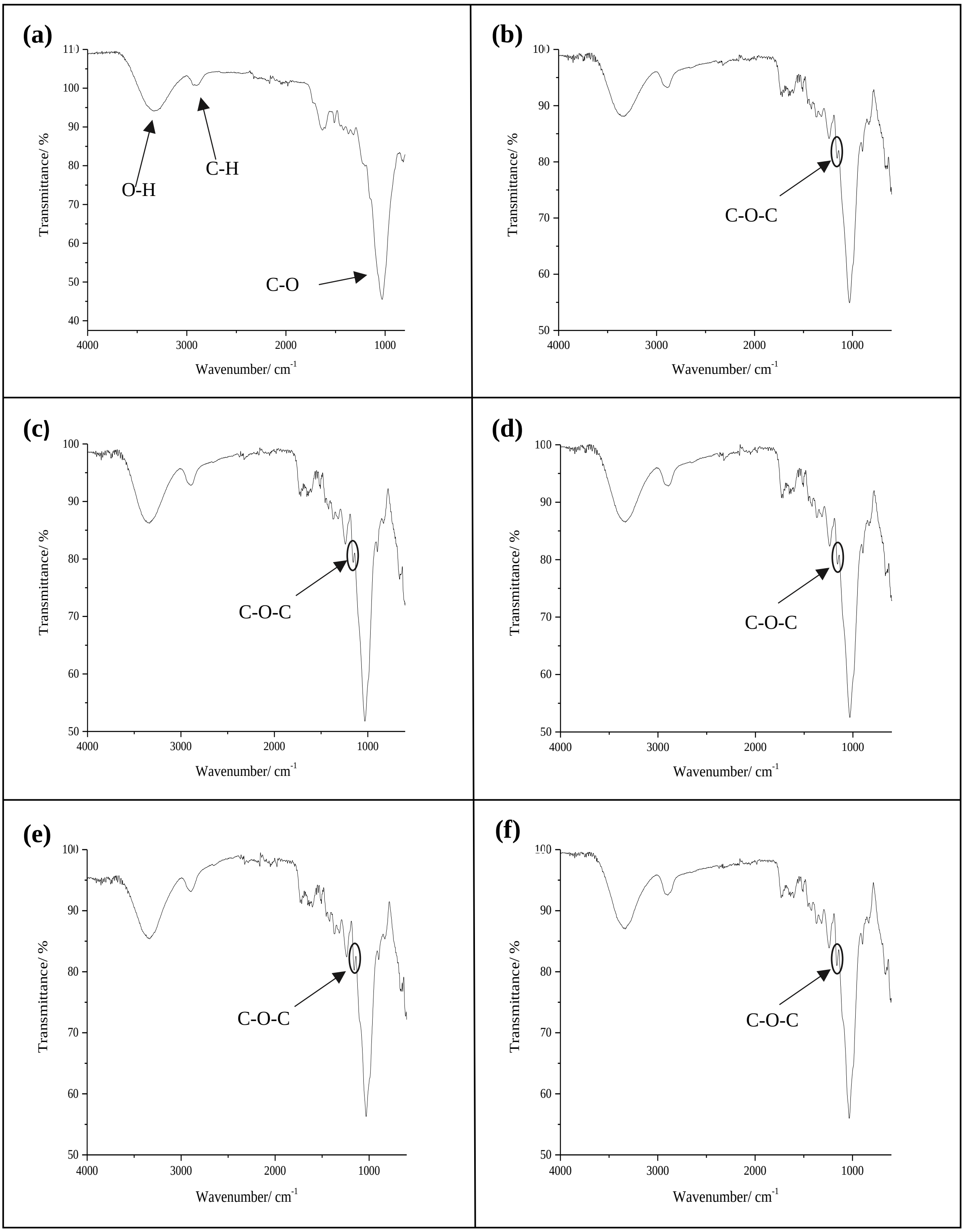


Figure 2. FTIR spectra of initial corn stover (a) and post reaction corn stover by the five ILs: (b) IL1, (c) IL2, (d) IL3, (e) IL4 and (f) IL5.

3.3. Cellulose extraction by imidazolium ILs

Figures 3 and 4 present pictures of microemulsion formed by $[C_{10}mim][(^iC_8)_2PO_2]$ and $[C_{12}mim][(^iC_8)_2PO_2]$, respectively. Figures 3A and 4A show the corn stover and IL just after the reaction. The solutions turned from light brown corn and transparent ILs to a brown gel form. Adding water caused the precipitation of the dissolved cellulose (Figures 3B and 4 B). Finally, hexane was added to form the microemulsion. Both mixtures were composed of four phases. Starting from the bottom there was post reaction corn stover (solid) with water, then the IL phase and on top, the transparent hexane phase (Figures 3C and 4C). One slight difference in the ILs behaviour was observed in that the volumes of IL phases differed for both mixtures. To check this, ^1H NMR was used with the three liquid phases for each experiment. It is of interest to see if there is ionic liquid in the aqueous phase and in the organic phase (hexane). The presence of ionic liquid in these phases represents a loss, and the procedure of separation of liquid phases should be improved. The spectra carried out in the aqueous and organic phases have not shown the presence of ionic liquid in both phases (spectra not shown). On the other hand, in the ionic liquid phases of $[C_{10}mim][(^iC_8)_2PO_2]$ and $[C_{12}mim][(^iC_8)_2PO_2]$, there were traces of water and hexane. These results do not allow to explain the decrease in the amount of ionic liquid in the system. ^1H NMR has shown that ionic liquid is not in the liquid or organic phases. On the other hand, traces of water and hexane have been found in the ionic liquid phase. The exact amount of these traces have not yet been determined and, will have to be done in the future.

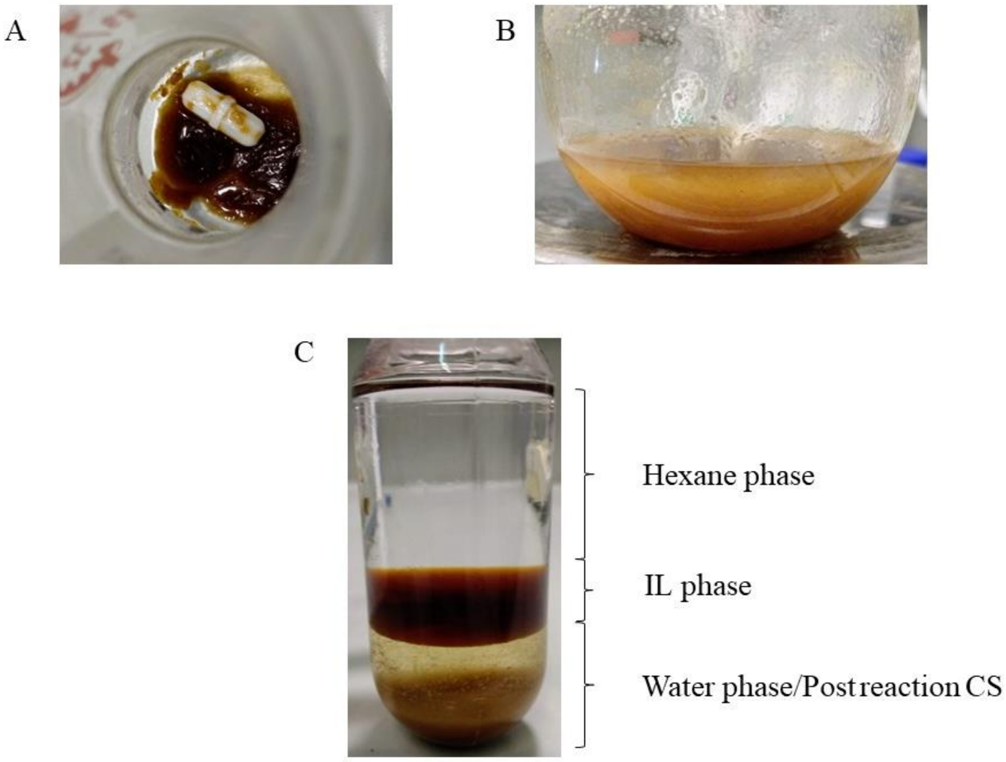


Figure 3. Microemulsion formation after reaction of $[\mathrm{C_{10}mim}][(^{i}\mathrm{C_8})_2\mathrm{PO_2}]$ and corn stover.

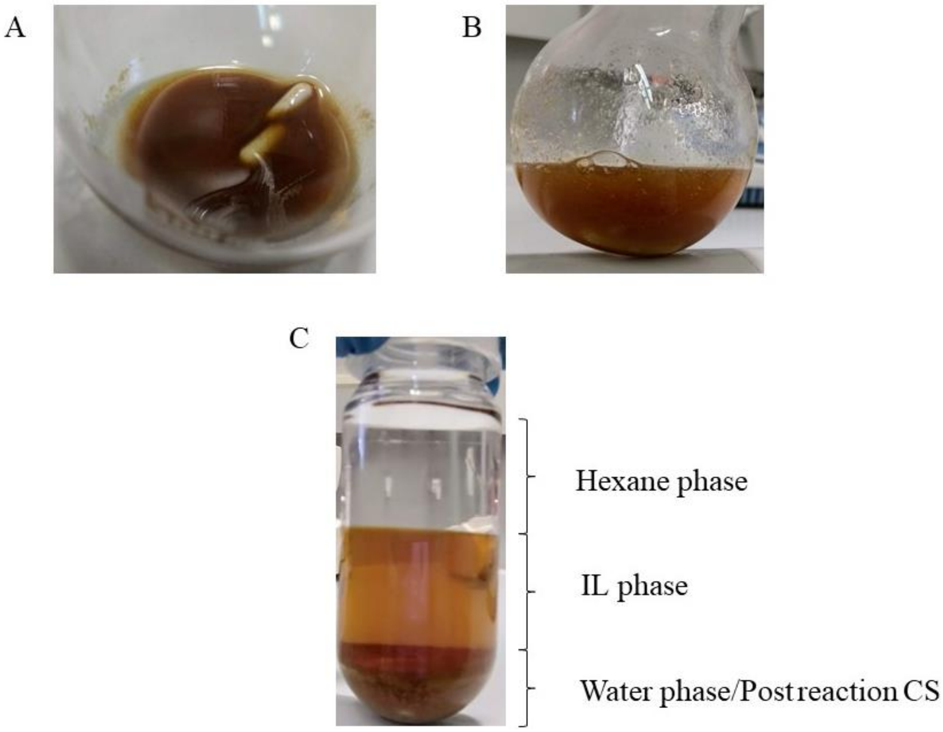


Figure 4. Microemulsion formation after reaction of $[\mathrm{C_{12}mim}][(^{i}\mathrm{C_8})_2\mathrm{PO_2}]$ and corn stover.

3.4. Reuse of imidazolium ILs

Recovered ILs were checked by ^{1}H NMR spectrum and their spectra were compared with the fresh ILs. As it can be observed in the Figure 5, the ILs after reaction with the corn stover are stable, no decomposition of their structure was observed. The same peaks were found for fresh and recovered ILs. Furthermore, recovered IL phases from the first reactions, without solvent evaporation (water, hexane) were applied again for cellulose extraction. In the case of the IL phase of $[C_{10}mim][(^iC_8)_2PO_2]$, the microemulsion were formed again after water and hexane addition (Figure 6a). On the contrary, for the IL phase of $[C_{12}mim][(^iC_8)_2PO_2]$, it was not necessary to add water and hexane. Two phases (IL/water with corn) formed after heating (Figure 6b). This happened because after the first reaction, the IL phase did not separate correctly and contained water and hexane, which in the next reaction helped to form microemulsion. The amount of ionic liquid lost in each cycle of extraction of cellulose was calculated. The average loss of IL/cycle were 20.5 ± 2.7% for $[C_{10}mim][(^iC_8)_2PO_2]$ and 12.6 ± 0.1 % for $[C_{12}mim][(^iC_8)_2PO_2]$.

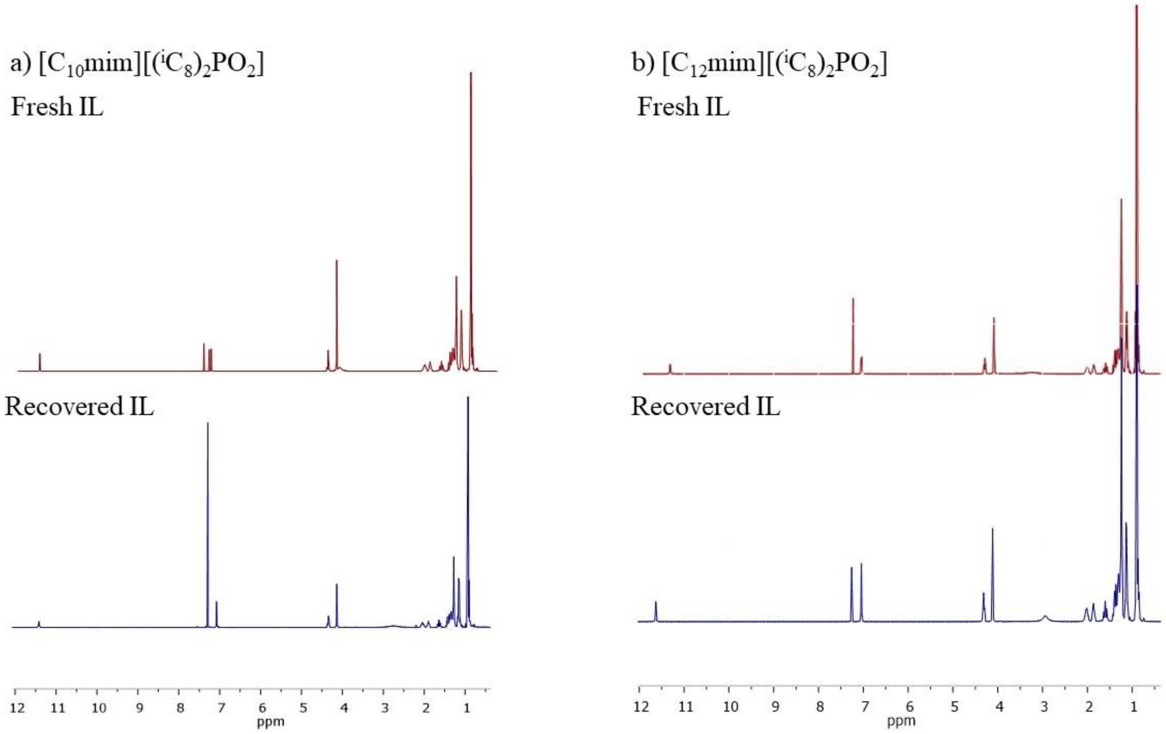


Figure 5. ^{1}H NMR of fresh and recovered ILs in $CDCl_3$ a) $[C_{10}mim][(^iC_8)_2PO_2]$, b) $[C_{12}mim][(^iC_8)_2PO_2]$.

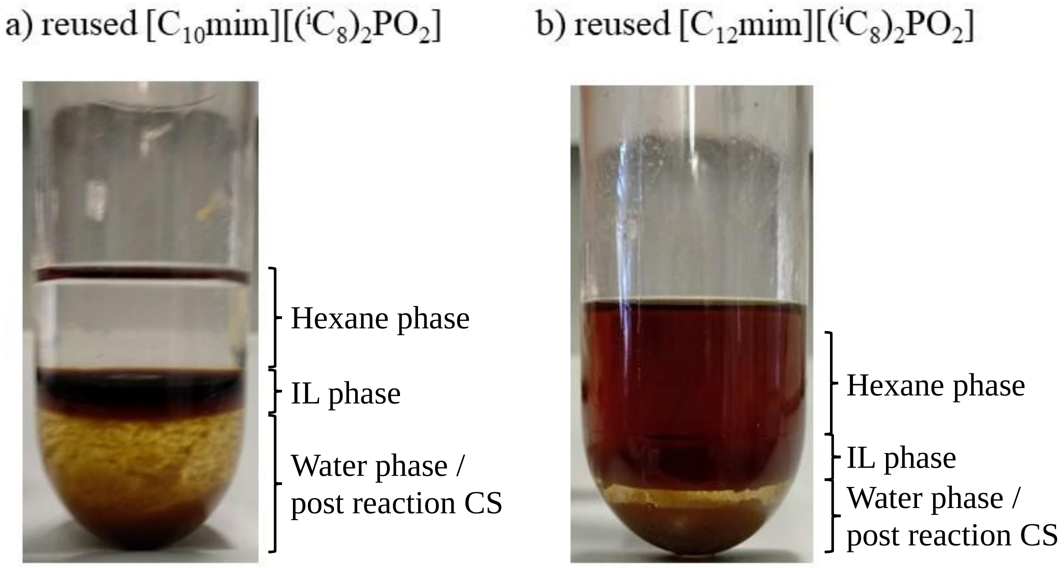
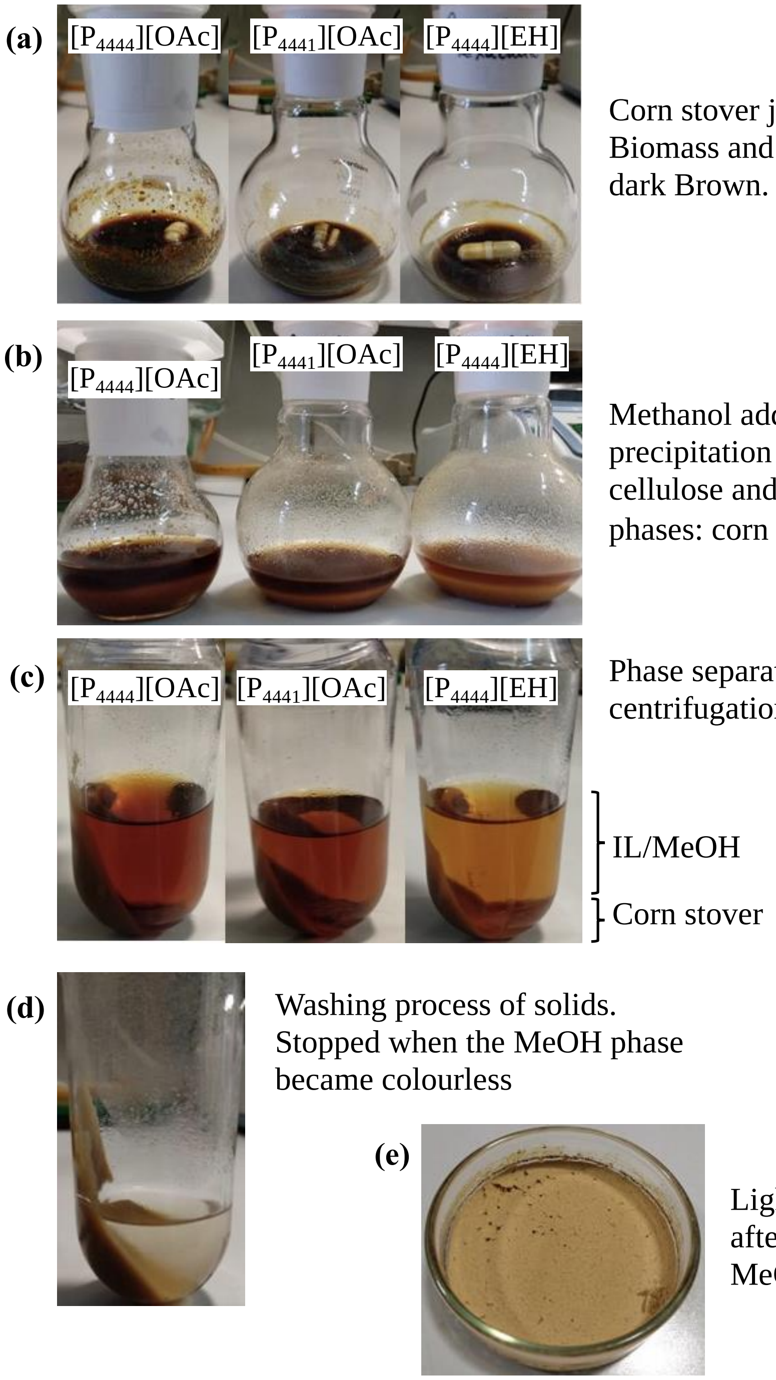

a) reused $[C_{10}mim][(^iC_8)_2PO_2]$ b) reused $[C_{12}mim][(^iC_8)_2PO_2]$

Figure 6. Recovered IL phases in again microemulsion formation.

3.5. Cellulose extraction by phosphonium ILs

Figure 7 presents the different stages of cellulose extraction from the corn stover by phosphonium ILs. Figure 7a shows the corn stover just after 3 h of reaction, when the biomass and colourless ILs mixtures turned to dark brown. Methanol addition (Figure 7b) caused the precipitation of the dissolved cellulose and the formation of two phases: corn stover and IL/MeOH. As it can be observed, the IL phase of the tetra-butyl-phosphonium 2-ethyl-hexanoate, $[P_{4444}][EH]$, produced a less intensive colour than the two other ILs. The phase separation was performed by centrifugation (Figure 7c) and by further washing of the solids with more MeOH. The washing process was stopped when the MeOH phase became colourless (Figure 7d). Then, the solvent (MeOH) was evaporated, resulting in a light brown precipitate (Figure 7e). All three phosphonium ILs were completely miscible with water, thus they were not recovered and reused. The recovery of those ILs would require an evaporation step (which is energy-consuming) and this does not coincide with the aim of the work.



Corn stover just after 3 h of reaction. Biomass and colourless Ils turned to dark Brown.

Methanol addition caused the precipitation of the dissolved cellulose and the formation of two phases: corn stover and IL/MeOH.

Phase separation obtained by centrifugation.

Washing process of solids. Stopped when the MeOH phase became colourless

Light brown precipitate after evaporation of MeOH.

Figure 7. Extraction steps with phosphonium ILs.

3.6. Characterization of extracted cellulose

Table 1 also shows the characterisation of products after the extraction of cellulose. As it can be observed, the total (%) of each product is lower than in the initial sample. This is because the amounts of hemicelluloses, glucose, lignin and ash in the products are quite different than in the initial sample. For all 5 ILs, these amounts are smaller and in a case of reused imidazolium

ILs are even smaller. Also, differences in the quantity of sugars soluble in water were found. In all cases, the amounts are lower than in the initial sample. The smaller amount of carbohydrates in products signifies that all 5 ILs are able to dissolve cellulose from corn stover however, the part of carbohydrates remains dissolved in ionic liquid. Additionally, the ILs remove part of the ashes leaving the corn stover cleaner.

In Table 3, the amounts of recovered cellulose for each IL and the ash content of products are also shown. Additionally, the amounts of recovered cellulose using recovered imidazolium ILs are included too. As it can be seen in the table, all ILs were able to dissolve cellulose in the presence of lignin despite the apparent difficulty to break the cell walls.

Tetra-butyl-phosphonium 2-ethyl-hexanoate (IL5) was the only IL able to extract 84% of the available cellulose, followed by, 1-Dodecyl-3-methyl-imidazolium bis-(2,4,4-tri-methyl-pentyl) phosphinate that was able to recover a significant amount of cellulose, 61%. The other ILs performances were very similar, between 43 to 50%. These results demonstrate that the design of the ionic liquids have a big importance on the performances of the dissolution of cellulose. The reason that IL 5 and IL 1 work the best in recovering cellulose is that they both have surface-active anions that in combination with water (emulsification) facilitates solubilisation of corn stover and further extraction of cellulose. These are more hydrophobic ionic liquid than the phosphonium shorter chained ones in the paper. They act as surfactants and help solubilisation of biomass (Hejazifar et al., 2016; Lins et al., 2018). It is also assumed that viscosity of ionic liquids has influence on biomass dissolution (Rieland and Love, 2020). Lower viscosity results in better mass transfer and therefore better contact of the components in the mixture and faster kinetics. For that reason, tetra-butyl-phosphonium 2-ethyl-hexanoate dissolves more cellulose than 1-dodecyl-3-methyl-imidazolium bis-(2,4,4-tri-methyl-pentyl) phosphinate. However, this is only a hypothesis, the viscosity of ionic liquids has not been measured.

Table 3. Recovered cellulose and ash content after reaction with ILs.

	Corn stover	**Recovered cellulose (%, w/w_{TS})**						
		IL1	IL2	IL3	IL4	IL5	reused IL1	reused IL2
Cellulose	35.2 ± 0.5	21.5 ± 3.4	15.5 ± 1.1	17.6 ± 0.0	15.1 ± 0.0	29.6 ± 0.0	26.9 ± 5.4	10.2 ± 0.0
		Cellulose recovery (%)						
		61.1	44.0	50.0	42.9	84.1	76.4	29.0
Ash	5.1 ± 0.0	**Ash content after reaction (%, w/w_{TS})**						
		3.4 ± 0.0	2.7 ± 0.0	4.3 ± 0.9	4.3 ± 0.9	4.1 ± 0.0	3.0 ± 0.0	2.3 ± 0.8
		Ash elimination (%)						
		33.3	47.1	15.7	15.7	19.6	41.2	54.9

Values are means ± SD, n = 3

On the other hand, the results in Table 3 showed that the $[P_{4444}][CH_3(CH_2)_3CH(C_2H_5)COO]$ (84.1%) has better cellulose recovery than $[P_{4444}][CH_3COO]$ (50%), which have the same cation but different anions. This can be explained by the fact that the anion of the ionic liquids acts as a hydrogen bond acceptor which interacts with the hydroxyl group of cellulose. Generally, the higher the hydrogen bond basicity of the anion, the greater ability to dissolve cellulose (Hou et al., 2015). The Kamlet-Taft parameter of hydrogen bond basicity β of $[P_{4444}]$[OAc] found in the literature was 1.36 (Xu et al., 2014) whereas Kamlet-Taft parameter β of $[P_{4444}]$[EH] could not be found. However, the Kamlet-Taft parameter of hydrogen bond basicity β of tetra-butyl-phosphonium hexanoate $[P_{4444}]$[Hex], 1.41, was found in the literature (Abe et al., 2015). The structure of $[P_{4444}]$[Hex] is very similar than the structure of $[P_{4444}]$[EH]. Then, the value of hydrogen bond basicity β of $[P_{4444}]$[Hex] could be attributed to $[P_{4444}]$[EH]. Assuming this hypothesis, The better recovery of cellulose by $[P_{4444}]$[EH] than $[P_{4444}]$[OAc] can be explained based on the β values, where higher value implies higher basicity.

Cellulose recovery results are very similar than observed in other works. Indeed, Zhang and co-workers (Zhang et al., 2015), working with ionic liquids based on imidazolium, [HMIM][Cl], [HMIM][HSO_4], [HMIM][CH_3COO], [HMIM][H_2PO_3], [HMIM][CH_3SO_3] and [HMIM][$C_7H_7SO_3$], have obtained values of recovery of cellulose between 40.0 and 76.6%. The best result being obtained with [HMIM][H_2PO_3], working at 70ºC for 3 h with ultrasound radiation (400 W) and with a solid:ionic liquid ratio of 1:20 (g:g). On the other hand, Xu and collaborators (Xu et al., 2016), working with deep eutectic solvents based on choline chloride and hydrogen donors, including urea, glycerol, formic acid, acetic acid, oxalic acid, malonic acid and citric acid, have obtained recoveries of up to 95.1% of the cellulose. Calculation of cellulose recovery was made from the glucose concentration after hydrolysis of the cellulose obtained. The experimental conditions used in this case were: temperature 130ºC for 2 h with mechanical agitation (100 rpm) and solid:deep eutectic solvent ratio of 1:20 (g:g). Finally, Liu and co-workers (Liu et al., 2018) carried out the pre-treatment of the corn stover with a mixture of an imidazolium based ionic liquid with a dissolution of sodium hydroxide. Agreeing to the authors, the mixture allows to obtain a greater recovery of cellulose. According to the operating conditions (temperature, time and solid ratio: ionic liquid), the recovered cellulose value varies between 31 and 89%. The best result being at 100ºC for 1.5 hours and with a solid:ionic liquid ratio of 1.0: 9.5.

The reuse of imidazolium ionic liquids has given contradictory results. The recovered ionic liquid [C_{10}mim][($^iC_8$)$_2PO_2$] has made it possible to extract 76.4% of the cellulose in a new experiment. The fresh ionic liquid had allowed to extract 61.1% of the cellulose. The increase in cellulose is considerable. According to another study, to carry out an efficient extraction of cellulose, it is necessary that the ionic liquid contains a small but sufficient amount of water (Abe et al., 2015). The recovered ionic liquid contains traces of water that have not been able to be eliminated. These traces of water have surely enhanced the dissolution of the cellulose.

On the contrary, the recovered ionic liquid [C_{12}mim][($^iC_8$)$_2PO_2$] has allowed to extract only 29.0% of the cellulose in a new experiment. The fresh ionic liquid had allowed to extract 44.0% of the cellulose. The decrease is very noticeable. In this case, the traces of water have not allowed to increase the extraction. And this is surely due to the presence of hexane in the ionic liquid that has inhibited the extraction. Although it cannot be seen after the regeneration of the ionic liquid, the amount of hexane must be considerable. These results show that the process of regeneration of the ionic liquid must be improved.

But, regarding ashes, a high quantity was still contained in the post reaction corn stover. Phosphonium ILs, were able to eliminate only ~ 17% of ashes. In fact, these phosphonium ILs are only able to recover cellulose, cleaning of the cellulose was not performed. On the contrary, the imidazolium ILs were able to remove 48% of the ashes from the solid phase. None of the studies commented before have presented results on the removal of ash from biomass. This does not seem to be of great importance for further processes of transformation of cellulose.

The proposed technology presents great technological challenges with a great economic implication before it can be scaled for an industrial application. First, the extraction efficiency is not still stable. $[C_{12}mim][(^{i}C_8)_2PO_2]$ allowed a better recovery after reutilisation, 76.4 instead 61.1%. On the contrary, $[C_{10}mim][(^{i}C_8)_2PO_2]$ presented worst results after reutilisation, 29.0 instead 44.0%. The instability can be justified by the residual amounts of water, hexane and ashes in the recovered ILs. The first big challenge is the design of improved ionic liquids able to solubilize the cellulose and remove all other impurities, ashes included. The cost of the extraction process of cellulose depends mainly on the cost of ionic liquids, due to their expensive cost of synthesis. This shows that the second most important challenges would be the production in large quantities of ionic liquids with an industrial cost. On the other hand, the relationship between corn stover and ionic liquid is very unfavourable for this last one. Industrial scaling of the process would now require huge volumes of ionic liquid that are not currently possible. To precipitate the cellulose, the mixture is mixed with a large amount of water. Therefore, it is of great importance to be able to reuse ionic liquids and not have losses during the regeneration process. This implies that firstly, a process unit has to be capable of eliminating the remaining ash in the ionic liquid. The third major challenge being the design of a continuous ionic liquid recovery process to separate the water. The removal of the water to attain the concentration initially present in the ionic liquid cannot be done by membrane separation processes. Works have shown that it is very difficult to concentrate the ionic liquid above 30 % (Haerens et al., 2010). The distillation of the mixture water-ethanol-ionic liquid is a possible solution to concentrate the ionic liquid, possible because ionic liquids have a boiling point which allows the distillation of the mixture. With all these conditions, stable extraction efficiency will be attained, making process attractive.

4. Conclusions

Five synthesised ILs with different structure were designed and synthesised in the laboratory. The ILs showed the ability to break cell walls and extract cellulose from the corn stover.

However, only two of them, the imidazolium ILs, can be considered as easily recovered due to their abilities to form microemulsions and being separated. In addition, imidazolium ILs turned out to be superior for removing ash from the stover. On the contrary, phosphonium ILs were more efficient in the recovery of cellulose but they were not able to remove ash from corn stover. Above all, these ILs needed an evaporation step, before to be recovered and reused.

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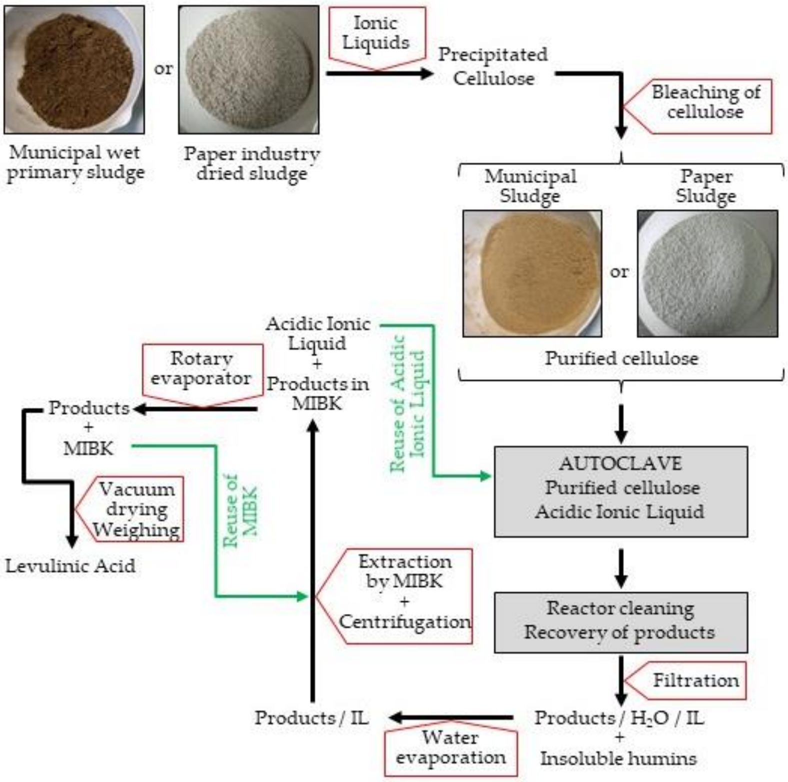
Chapter 6

Valorization of cellulose recovered from WWTP sludge to added value levulinic acid with a Brønsted acidic ionic liquid [1]



ABSTRACT

The progressive decline of using fossil sources in the industry means that alternative resources must be found to produce chemicals. Waste biomass (sewage sludge) and waste lignocellulosic resources (food, forestry or paper industries) are ideal candidates to take over from fossil sources. Municipal sewage sludge, and especially primary sludge, has a significant proportion of cellulose in its composition. Proper treatment of this cellulose allows the production of interesting chemicals like levulinic acid, that are precursors (bio-blocks or building blocks) for other organic chemical processes. Cellulose was extracted from municipal wet primary sludge

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[1] Glińska K., Lerigoleur C., Giralt J., Torrens E., Bengoa C. Valorisation of cellulose recovered from WWTP sludge to added value levulinic acid with a Brønsted acidic ionic liquid. *Catalysts* 10 (2020) 1004.

and paper industry dried sludge with a commercial ionic liquid. More than 99% of the cellulose has been recovered in both cases. Extraction was followed by the bleaching of the cellulose for its purification. In the bleaching, a large part of the ash was removed (up to 70% with municipal sludge). Finally, the purified cellulose was converted in levulinic acid by catalyzed hydrothermal liquefaction. The reaction, done at 170ºC and 7 bar, catalyzed by a tailored Brønsted acidic ionic liquid produced levulinic acid and other by-products in smaller quantities. The process had a conversion of cellulose to levulinic acid of 0.25 with municipal sludge and of 0.31 with industrial sludge. These results fully justify the process but, require further study to increase the conversion of cellulose to levulinic acid.

1. Introduction

The fossil sources are non-renewable and, will be depleted in near future. According to International Energy Agency, coal reserves will be available up to 2112 while easy production of oil and gas will be spent by 2040 and 2042, respectively. Therefore, scientists are exploring how to produce chemicals from biomass sources in order to meet the human needs. There are 12 building blocks, or chemical platforms, essential molecules which can be converted to a wide range of chemicals or materials. These building blocks can be obtained from waste biomass without exposing supplies like food, feed, forests and in general, biodiversity of the world. Moreover, the valorization of the waste biomass is beneficial from a point of view of sustainable waste management. Additionally, the valorization is economically attractive by eliminating waste disposal fees and giving value to wastes [1].

Building blocks are the basic components for organic chemistry. One of the building blocks is the levulinic acid (LA). LA is used as a building block for fuel additives, solvents, flavor substances, pharmaceutical agents, coating, dyes, rubber and plastic additives and other industries [2,3]. The levulinic acid can be obtained from cellulose contained in biomass sources [4].

The production of levulinic acid from lignocellulosic material by conventional methods require the use of inorganic mineral acids (e.g., H_2SO_4, HCl, H_3PO_4 and HBr) and metal salts [5-6]. These catalytic combinations have the disadvantage of causing equipment corrosion, environmental problems and difficulty with recyclability [7-9]. In turn, solid acid catalysts are an environmentally friendly replacement for liquid acid catalysts. They are recoverable and recyclable despite that the obtained yield is low and they need long reaction times [9-10]. Ionic liquids (ILs) are used as solvents to dissolve the cellulose. They can also be used as catalysts in the reaction to produce levulinic acid. In comparison with conventional methods, ILs are characterized by high thermal stability, low corrosive nature, easy recoverability, low vapour pressure and easy functionalization with high acidity [11]. In this study, ionic liquid with functional group ($-SO_3H$) was used for the catalytic conversion of cellulose to levulinic acid. According to the literature [11], the catalytic activity of the functionalized ionic liquids decreased in the following order: $IL-SO_3H > IL-COOH > IL-OH$. Furthermore, the type of the cation has an effect on the acidity of IL. It is shown [12], that Brønsted acidic ILs with imidazolium-based cation has better catalytic activity than ammonium or triphenylphosphonium-based cations.

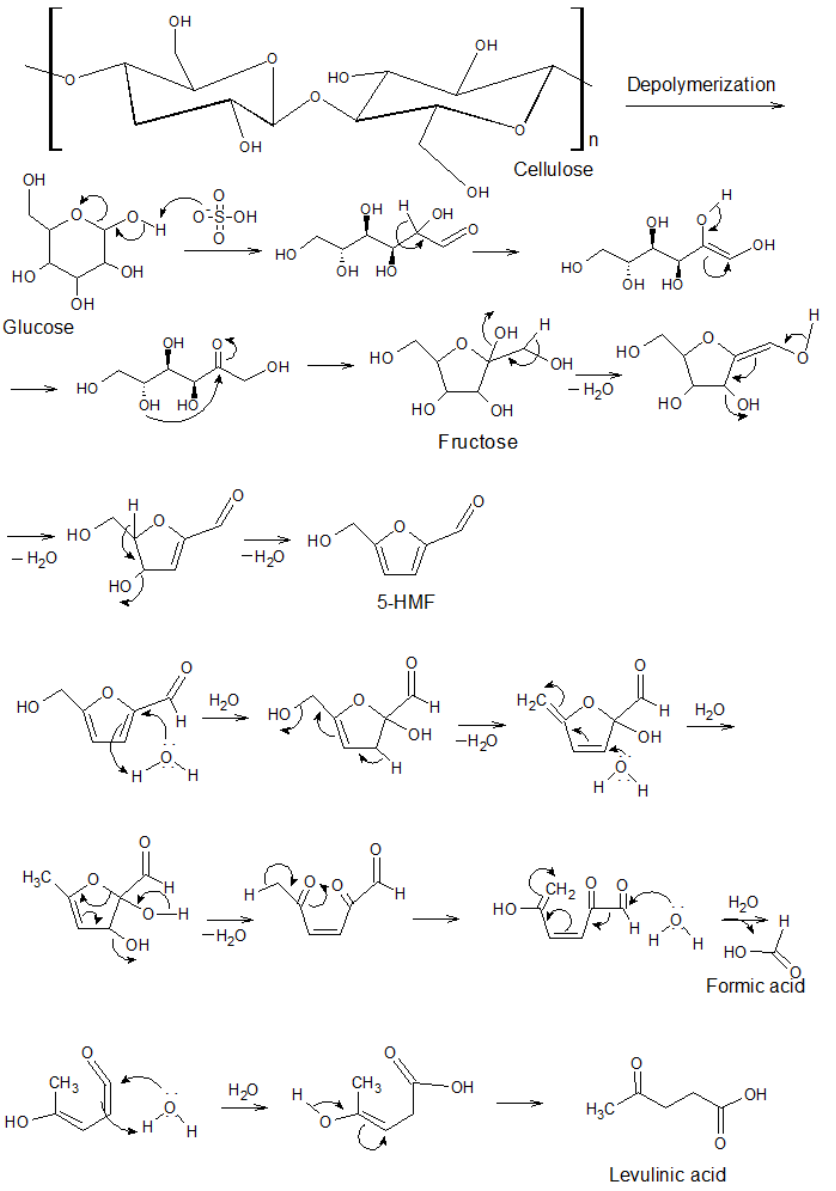

Figure 1. Mechanism of conversion of cellulose recovered from sludge to levulinic acid catalyzed by Brønsted acidic ionic liquid (adapted from Badgujar et al., 2019 [13]).

In the first step, the cellulose is depolymerized into the monomeric gluco-pyranose sub-units. The acidic group of IL (sulphonic acid) causes the formation of a carbonyl group by the opening of pyranose ring. The carbonyl group goes through the keto-enol equilibrium and generates the 1,2-enediol. Next, the 1,2-enediol via a ring closure reaction produces fructose. Then, the keto-enol isomerization of fructose gives the 5-hydroxymethylfurfural (5-HMF) and 3 molecules of water are eliminated [13]. During the rehydration reaction of 5-HMF, levulinic acid is formed while a parallel reaction is forming a by-product: insoluble polymeric humins [3,14]. Humins are insoluble macromolecular components remained after carbohydrates catalysis. They can be used as composite material, building blocks or soil amendment [15-17].

According to the literature [18], during the catalytic conversion of cellulose to levulinic acid, 5-HMF is obtained as by-product and, its concentration increases at reaction temperatures above 200ºC. Other by-products that can be obtained are furfural, formic acid and acetic acid. Due to the hydrolysis of hemicellulose to xylose followed by its dehydration allows the formation of furfural. A further dissociation of furfural let to obtain formic acid and acetic acid [19].

So far, in our view, only two papers have been published related to the production of levulinic acid from sludge or residual biomass from the paper industry [20,21]. In the first work, paper mill sludge was obtained from a packaging company and converted by hydrothermal liquefaction catalyzed by hydrochloric acid. Tests were also carried out with a microwave reactor. The conversion in levulinic acid obtained have approached 80% of the theoretical [20]. In the second work, fiber sludge from Finnish and Swedish pulp industry has been used. In this case, a microwave reactor was used, the reaction being catalyzed by Bronsted and Lewis acids. In this case, the conversion has not exceeded 35% with respect to the theoretical [21].

There are three main objectives in this work: (i) recovery of cellulose from waste biomass: municipal wastewater treatment plant (WWTP) primary wet sludge and paper industry dried mixed sludge, from primary treatment and membrane bioreactor purge. This first part was done using well-known methodology from previous studies [22-24]; (ii) the recovered cellulose from both type of sludges was purified by a bleaching procedure to decrease the amount of proteins and ashes in the solid phase after extraction; (iii) the extracted and purified cellulose was converted to levulinic acid, value-added chemical considered as building-block by chemical industry. Hydrothermal liquefaction catalyzed by Brønsted acidic ionic liquid was used to produce the levulinic acid.

To the best of our knowledge, this is the first time that: (i) cellulose was recovered from municipal and industrial sludge by extraction with commercial ionic liquids; (ii) the recovered cellulose was bleached in two steps; (iii) the purified cellulose was converted in levulinic acid by hydrothermal treatment catalyzed by home-made ionic liquid, all processes done consecutively.

2. Results and discussion

Figure 2 presents the overall scheme of the process of production of levulinic acid from cellulose. In a first step, the cellulose is recovered from WWTP primary wet sludge and paper industry dried mixed sludge, from primary treatment and membrane bioreactor purge. The extraction was realized with Tetrakis ionic liquid [22-24]. In a second step, the recovered cellulose was bleached through two consecutives treatments with hydrogen peroxide and hydrochloric acid. Finally, purified cellulose was treated by catalyzed hydrothermal liquefaction to produce levulinic acid. Figure 1 also shows all the procedures of separation of products and their characterization after the overall process.

2.1. Characterization of sludge

Primary sludge was collected from the municipal WWTP of Reus (Tarragona, Spain). The WWTP has a capacity to daily process 25,000 m^3 of wastewater. Primary sludge was collected after the partial gravity thickening, located later the primary decanter. The industrial sludge was provided by the paper international company Gomà-Camps, S.A. situated in La Riba (Tarragona, Spain). This sludge is a mixed sludge from physical-chemical primary treatment and purge of membrane biological reactor. Both effluents are mixed and dried with hot air.

As received, both sludges differ a lot, municipal WWTP primary sludge, henceforth municipal sludge, is essentially a liquid with solids in suspension. On the other hand, paper industry dried sludge, henceforth industrial sludge, is a solid. This difference is essentially due to the water content of each sludge. The results of the characterization of both sludges are presented in Table 1.

As it can be seen in the table, the moisture of municipal sludge reaches 96.2% whereas in industrial sludge this value is almost half-lower: 53.1%. Then, the total solids are 3.8% and 46.9% for municipal sludge and industrial sludge respectively. After drying, both sludges also differ in their composition. Municipal sludge is constituted by more or less 25% w/w_{TS} of inorganic ashes and 25% w/w_{TS} of each organic matter: lipids, carbohydrates and proteins. This composition has been quite constant during the last years as it can be seen comparing other works [22,25] and now. In contrast, industrial sludge is mainly constituted by ashes (50.5% w/w_{TS}) and carbohydrates (42.3% w/w_{TS}). The rest of the organic substances of the industrial sludge represent a low proportion: lipids (1.2% w/w_{TS}) and proteins (5.0% w/w_{TS}). This composition is similar than the observed in a recent work of the authors [24].

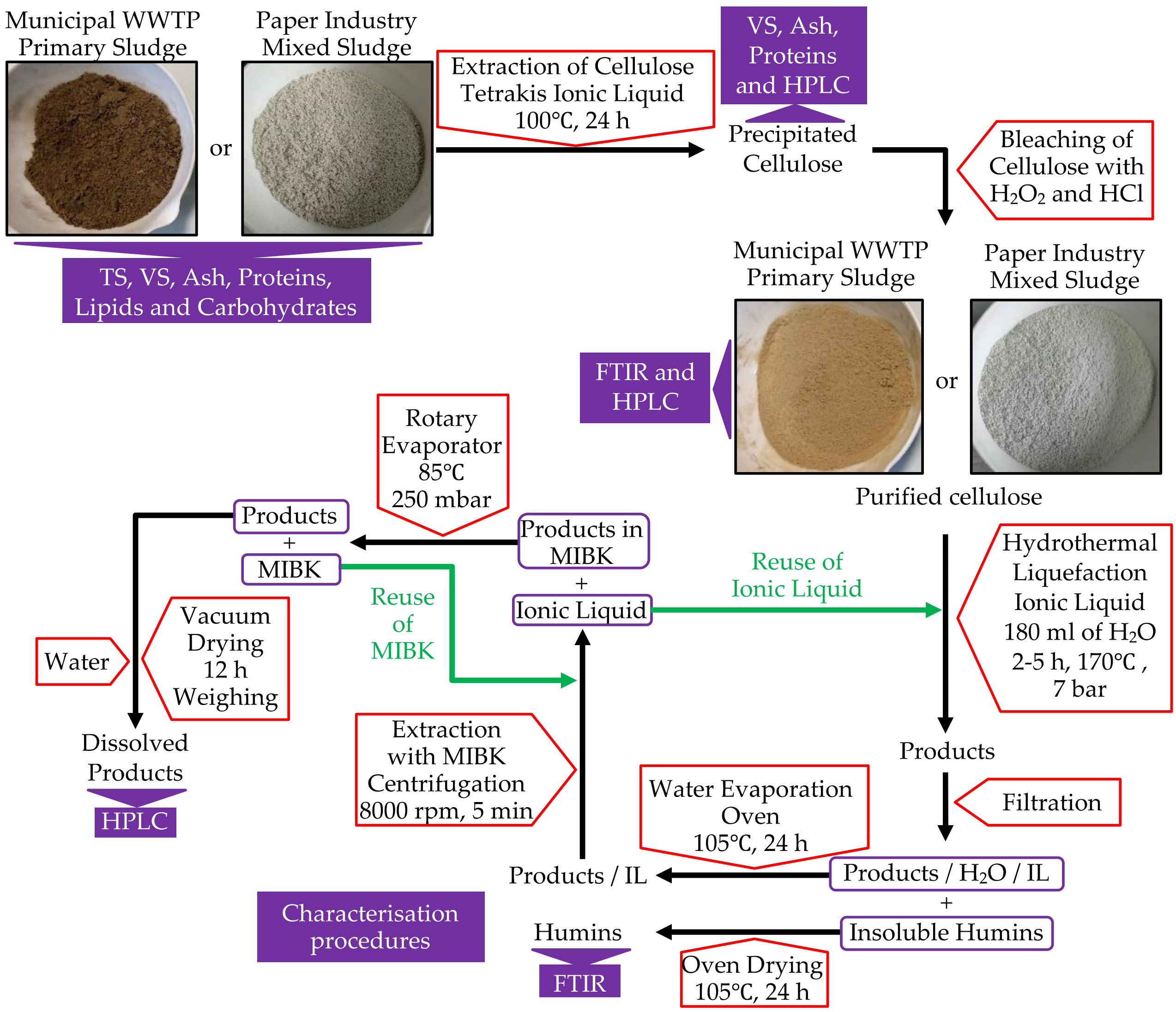

Figure 2. Scheme of full process of conversion of cellulose recovered from sludge to levulinic acid catalyzed by Brønsted acidic ionic liquid.

Table 1. Characterization of municipal primary sludge and industrial paper sludge by conventional procedures.

	Municipal primary sludge	**Industrial paper sludge**
TS (%, $w/w_{\text{wet sludge}}$)	3.8 ± 0.1	46.9 ± 1.1
Moisture (%, $w/w_{\text{wet sludge}}$) *	96.2 ± 0.1	53.1 ± 1.1
Ashes (%, w/w_{TS})	23.5 ± 2.3	50.5 ± 0.2
VS (%, w/w_{TS}) *	76.5 ± 1.7	49.5 ± 0.2
Lipids (%, w/w_{TS})	22.9 ± 1.8	1.2 ± 0.1
Proteins (%, w/w_{TS})	28.2 ± 1.5	5.3 ± 0.1
Carbohydrates (%, w/w_{TS})	25.8 ± 1.9	42.6 ± 0.7
Total (%, w/w_{TS})	100.4 ± 7.5	99.6 ± 0.9

Values are mean, ±SD, n=3.
* By difference.

2.2. Extraction of cellulose from both municipal and industrial sludges with IL

The process of recovery of cellulose from municipal and industrial sludges was performed using a similar method presented elsewhere [22-24]. Tetrakis ionic liquid was used to extract the cellulose. The temperature of process was fixed at 100ºC and the extraction was performed during 24 h. These values were optimized in the past [22-24]. Then methanol was added to the mixture to precipitate the cellulose. Three phases were obtained: (i) organic phase in the top of the flask; (ii) intermediate light brown aqueous phase containing ionic liquid, water, methanol, part of the proteins and part of the ashes; (iii) precipitated brown phase containing all cellulose and the rest of proteins and ashes. Then, hexane is added to the mixture to dissolve the organic phase and, is separated with a pipette. The operation was repeated until the organic phase is transparent. The aqueous phase and the precipitate were separated by centrifugation, 3500 rpm for 10 minutes. The precipitate containing cellulose and part of the proteins and ashes was separated by centrifugation at 6000 rpm during 10 min. The precipitate was cleaned with methanol and centrifuged again in the same conditions. Then the precipitate was characterized.

2.3. Characterization of the solid products after the process of recovery with IL

Table 2 presents the characterization of the solid products after the process of recovery of cellulose from both sludges. As it was expected, taking into account experimental errors, the process of recovery with Tetrakis IL was able to recover almost all carbohydrates from both sludges. However, besides of carbohydrates, the solids still contain a part of ashes and proteins.

Table 2. Characterization of the precipitates after the recovery of cellulose (100℃, 24-h).

Composition (%,w/w_{TS})	Municipal sludge	Industrial sludge
Proteins	8.9 ± 1.9	4.1 ± 0.7
Ashes	16.2 ± 2.5	33.6 ± 0.5
Carbohydrates	28.7 ± 5.3	43.5 ± 1.3
Total	53.8 ± 9.7	81.2 ± 2.5

Values are mean, ±SD, n=3.

On the contrary, lipids were totally removed during the separation. With municipal sludge, the process was able to decrease the quantity of ashes from 23.5 to 16.2% w/w_{TS} and the quantity of proteins from 28.4 to 8.9% w/w_{TS}. In the case of the industrial sludge, the drop-in values were from 50.5 to 33.6% w/w_{TS} and from 5.0 to 4.1% w/w_{TS} for ashes and proteins respectively. The presence of ashes and proteins in the solid products required the need to clean them to purify the cellulose.

2.4. Bleaching of precipitate

The procedure of the bleaching of recovered precipitate was based on the literature [26,27]. According to these works, hydrogen peroxide is commonly used as a bleaching agent responsible for lignin dissolution. In the first step of the process, a dried batch of recovered precipitate from municipal or industrial sludge was mixed with a solution of hydrogen peroxide (H_2O_2) 8% for the dissolution of lignin and protein. The mixture was stirred 24 h at room temperature. Then, the mixture was filtered. The precipitate was cleaned with distillate water. The content of protein and ashes were determined in both precipitates. The results of the bleaching process are presented in Table 3.

Table 3. Characterization of the precipitates after bleaching (8% H_2O_2 and 0.1 N HCl).

Composition (%,w/w_{TS})	**Municipal sludge**	**Industrial sludge**
Proteins *	6.4 ± 1.6 (-28%)	1.8 ± 0.4 (-56%)
Ashes **	4.9 ± 1.8 (-70%)	21.5 ± 0.3 (-36%)
Carbohydrates	24.4 ± 7.0 (-15%)	38.3 ± 3.7 (-12%)
Total	35.7 ± 10.4 (-34%)	61.6 ± 4.4 (-24%)

Values are mean, ±SD, n=3.

* Quantified after hydrogen peroxide treatment.

** Quantified after hydrochloric acid treatment.

In brackets rate of reduction.

Proteins were determined after H_2O_2 treatment. As it can be seen in the table, the H_2O_2 solution was able to dissolve and remove 28% and 56% of protein from municipal and industrial precipitates, respectively. Proteins dissolution in H_2O_2 can be explained by the dissociation of H_2O_2 to hydrogen (H+) and hydroxyl (OH-) radicals. Then, these oxidative agents readily attacked proteins and decomposed them into soluble amino acids [28]. But, H_2O_2 did not reduce the quantity of ashes from the precipitate, acid hydrolysis with 0.1N HCl is expected to do that.

In the second step, both precipitates were hydrolyzed with hydrochloric acid (HCl) 0.1 N during 5 h in an ultrasonic bath. The initial transparent acid dissolution changed color to light brown/yellow. After separation, cleaning and drying of precipitate, ashes were quantified. The acidic treatment was able to remove 70% and 36% of the ashes from municipal and industrial precipitates, respectively. It is noticeable that during the bleaching treatment a loose of carbohydrates was also observed, 15% and 12% from municipal and industrial precipitates, respectively. According to the literature [26], the hydrolysis also makes isolation of the pure cellulose fibers by hydrolyzing traces of hemicellulose and lignin to simple sugars. In Table 4 are presented the results of HPLC analysis of precipitates before and after bleaching. It can be seen in the table that the amount of hemicellulose and lignin reduced after bleaching. But, the amount of cellulose increased for both sludges, more in the case of the industrial sludge, from 32.8 to 42.3%. This increasing can be explained by the growth of the amount of simple sugars, such as glucose, provoked by the treatment with HCl. As the measure of cellulose is obtained from the values of glucose [29], the results show an increasing amount of cellulose.

Table 4. Characterization of the carbohydrates by HPLC after bleaching (8% H_2O_2 and 0.1 N HCl).

Carbohydrates (%, w/w$_{\text{precipitate}}$)	**Municipal sludge**		**Industrial sludge**	
	Before bleaching	After bleaching	Before bleaching	After bleaching
Hemicellulose	7.7 ± 1.0	3.4 ± 0.1	7.2 ± 0.1	6.5 ± 0.0
Cellulose*	25.3 ± 0.6	26.7 ± 3.2	32.8 ± 1.3	42.3 ± 0.9
Lignin	20.0 ± 3.0	17.7 ± 0.3	10.9 ± 2.3	10.2 ± 0.3

Values are mean, ±SD, n=3.

*Amount of cellulose calculated by division of the amount of glucose by a conversion factor of 1.11 [29].

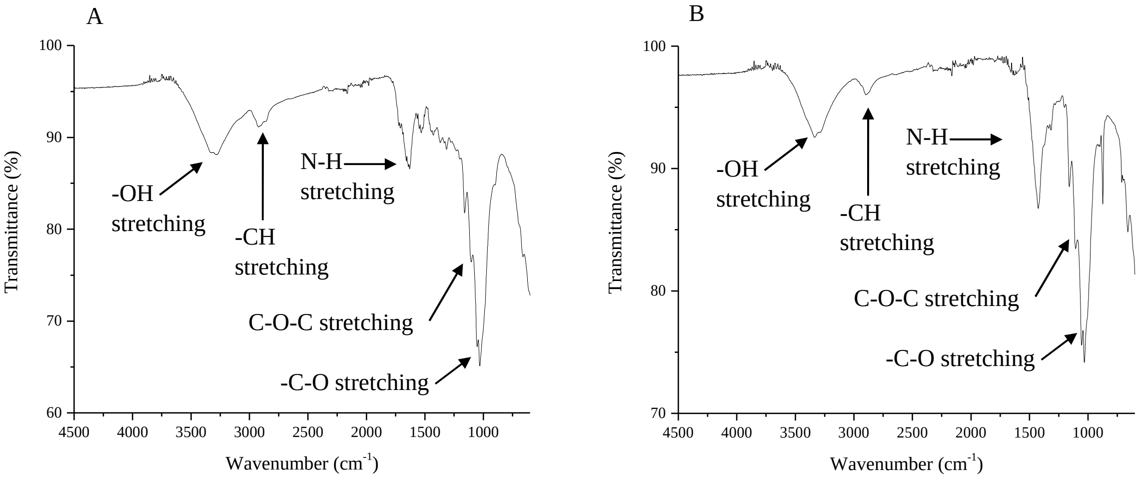

Figure 3. FTIR spectra of purified cellulose after extraction with ionic liquid and bleaching with H_2O_2 and H_2SO_4: (A) municipal sludge; (B) industrial sludge.

Figure 3 presents FTIR spectra of purified cellulose recovered from municipal sludge (a) and industrial sludge (b). Both spectra present some characteristic absorbances in different frequency regions: 3300 cm^{-1} of O-H group, 2900 cm^{-1} assigned as the CH_3 and CH_2 stretching vibration of cellulose, 1160 cm^{-1} of C-O-C stretching vibration particularly associated with cellulose and the broad peak 1030 cm^{-1} of C-O stretching vibration of carbohydrates. All those peaks demonstrate that purified solids contain cellulose. In Figure 3 (a) there is also presented the peak at 1650 cm^{-1} associated to peptide amide groups of proteins while in Figure 3 (b), is absent. That confirms that after bleaching some part of proteins (6.8%) stay in the cellulose recovered from municipal sludge whereas the amount of proteins in the purified cellulose recovered from industrial sludge is too small (1.8%) to be visible in the spectra.

2.5. Cellulose conversion to levulinic acid

The procedure of conversion of cellulose to levulinic acid catalyzed by Brønsted acidic ionic liquid was directly based on a recent work [3]. In this work, authors have synthesized and tested 9 different ionic liquids. The results showed that the acidity of the ionic liquid has the greater importance on the yield of the reaction. The acidity depends on the cation group and on the anion. The $[mimC_4SO_3H][HSO_4]$ ionic liquid has the higher acidity resulting in its design by the presence of the imidazolium group and by the hydrogen sulphate anion [3]. In consequence, $[mimC_4SO_3H][HSO_4]$ was selected and synthesized to convert cellulose to levulinic acid by catalyzed hydrothermal liquefaction.

After the reaction, the reactor was cleaned with deionized water and the products were separated and characterized. The first separation was realized by filtration. Residual solids were abundantly washed with deionized water, dried and weighed. The liquid phase is a mixture of water, ionic liquid and products. Water was evaporated overnight in an oven. Then, the ionic liquid was separated from the resulting liquid mixture by addition of methyl isobutyl ketone (MIBK). Two liquid phases were formed, the upper phase contain the levulinic acid dissolved in the MIBK while the lower phase the ionic liquid. Both phases were separated by centrifugation at 8000 rpm for 5 min. MIBK was evaporated from organic phase at the rotary evaporator at 85ºC and 250 mbar. Resultant products were dried under vacuum and weighed. Finally, products were characterized by HPLC. In Table 5 are presented the values of the weight of biomass, weight of cellulose contained in the biomass, weight of ionic liquid and volume of water used in each reaction. Table 5 also presents the values of weight of soluble in MIBK products, weight and percentage of levulinic acid and finally weight and percentage of humins.

As it was expected, levulinic acid was obtained in each reaction with the three sources of cellulose: pure from provider, from municipal and from industrial sludge. About the commercial cellulose, entries 1 to 3, it can be observed that with a reaction time of only 2 hours, the conversion to levulinic acid was only 18.8%. Increasing the reaction time to 5 hours allowed a conversion of 57.8%. This is normal, increasing the reaction time produces an increase in the conversion. However, this increase in conversion reaches a maximum and then decreases, due to the appearance of condensation and recombination reactions of the products. Then, it will be necessary in a future work to confirm this fact, because it is possible that optimized time of reaction was not attained. On the other hand, the decrease of the ratio between the weight of the ionic liquid and the weight of cellulose in the sample, $w_{IL}/w_{Cellulose}$: from 6.67 to 1.88, allowed to increase the conversion to levulinic acid until 81.3%. The effect of the ratio $w_{IL}/w_{Cellulose}$ on the conversion to levulinic acid seems to be important. This will be studied in more detail in further work.

Table 5. Weight of products, levulinic acid and humins after hydrothermal treatment of cellulose with acidic ionic liquid (temperature: 170ºC, time of reaction: 5 h).

Nº	**Source**	**Biomass (g)**	**Cellulose (g)**	**IL (g)**	**H_2O (mL)**	**Products (g)**	**Levulinic acid**		**Humins**	
							(g)	**(%)**	**(g)**	**(%)**
1	Sigmacell*****	-	4.8	30.0	180	0.9	0.9	18.8	3.2	66.7
2	Sigmacell	-	4.5	30.0	180	2.7	2.6	57.8	0.9	20.0
3	Sigmacell	-	16.0	30.0	180	13.1	13.0	81.3	3.0	18.8
4	IS Purified	6.7	2.8	26.6	180	0.4	0.2	7.1	5.9	88.1
5	IS Purified	8.9	3.8	26.6	180	0.6	0.4	10.5	8.3	93.3
6	IS Purified	9.8	4.2	30.0	180	1.4	1.3	31.0	8.8	89.8
7	IS Purified	8.9	3.8	36.0	180	0.7	0.7	18.4	7.6	84.4
8	MS Purified	8.2	2.2	26.0	180	0.6	0.5	22.7	6.8	82.9
9	MS Purified	19.9	5.3	36.0	180	0.7	0.6	11.3	14.2	71.4
10	MS Purified	8.9	2.4	26.0	180	0.7	0.6	25.0	8.0	89.9
11	IS	12.1	4.1	26.0	180	0.01	0.01	0.2	10.2	84.3
12	MSar	200.9	1.0	6.0	0	0.03	0.02	2.0	6.8	89.5

* 2h reaction; **IL**: Ionic Liquid ; **IS**: Industrial Sludge (as received); **MS**: Municipal Sludge; **MSar**: Municipal Sludge (as received, wet, TS: 3.8% $w/w_{wet\ sludge}$).

The results of conversion to levulinic acid obtained with industrial sludge are less good (entries 4 to 7). In the four experiments carried out, the conversion to levulinic acid was between 7.1 and 31.0%. The reduction of the conversion is normal since in the samples of industrial sludge there is presence of hemicellulose, lignin, ash and other materials that disturb the reaction of conversion of the cellulose. As it was the case with commercial cellulose (Sigmacell), the decrease in the ratio $w_{IL}/w_{Cellulose}$ causes an increase in the conversion to levulinic acid. Indeed, in entries 4 and 7 the ratio is 9.5 and the conversions are 7.1 and 18.4% respectively. In entries 5 and 6 the ratio is 7.0 and conversions are 10.5 and 31.0% respectively. However, there is a disparity between the results obtained with the same ratio. There is another variable that seems to be important: the water to cellulose ratio, $w_{Water}/w_{Cellulose}$. Indeed, in entries 4 and 7 the $w_{Water}/w_{Cellulose}$ ratios were 64.3 and 47.4 respectively. On the other hand, in entries 5 and 6 the $w_{Water}/w_{Cellulose}$ ratios were 47.4 and 42.9 respectively. The decrease in the $w_{Water}/w_{Cellulose}$ ratio causes a consequent increase in the conversion to levulinic acid. These trends will have to be confirmed in subsequent work.

In the case of municipal sludge, entries 8 to 10, the results of conversion to levulinic acid were quite similar: between 11.3 and 25.0%. Again, impurities in the samples were responsible of the decrease of the conversion to levulinic acid. But with municipal sludge, the decrease in the ratio $w_{IL}/w_{Cellulose}$ does not seem to present a clear trend. Indeed, in entries 8, 10 and 9, the $w_{IL}/w_{Cellulose}$ ratios are 11.8, 10.8 and 6.8 respectively, while the conversions to levulinic acid are 22.7, 25.0 and 11.3% respectively. It can be seen that between entries 8 and 10 there is an increase in the conversion but, then, it decreases sharply. The effect of the $w_{Water}/w_{Cellulose}$ ratio on the conversion was the same. In entries 8, 10 and 9, the $w_{Water}/w_{Cellulose}$ ratios are 81.8, 75.0 and 34.0 respectively. The explanation for this negative result must be sought in the design of the experiment. Indeed, the experiment in entry 9 was carried out with a mass of sludge of almost 20 g, while in experiments 8 and 10 this mass was between 8 and 9 g. While the amount of cellulose available is higher, the amount of impurities even more. This must have disturbed the cellulose conversion reaction and maybe the mixing into the reactor. On the other hand, observing the mass balances of these experiments it can be seen that the balance for entry 9 was only 75%, while the mass balances for entries 8 and 10 were 90 and 98% respectively. This fact could explain the bad result obtained with the experiment in entry 9.

The experiments realized with industrial and municipal sludge as received, entries 11 and 12 respectively, gave even smaller conversions to levulinic acid: 0.2 and 2.0% respectively. The cause of this great decrease can be found in the fact that both sludges contain large amounts of ashes, 23.5 and 50.5% respectively.

As it can be seen in Table 5, levulinic acid represents more of the 90% of the total products. The other products are lactic acid, formic acid, HMF and furfural. These other products were also found elsewhere [3]. In Figure 4 are presented two chromatograms from HPLC analysis of products obtained from hydrothermal processing of cellulose from industrial sludge (a) and municipal sludge (b).

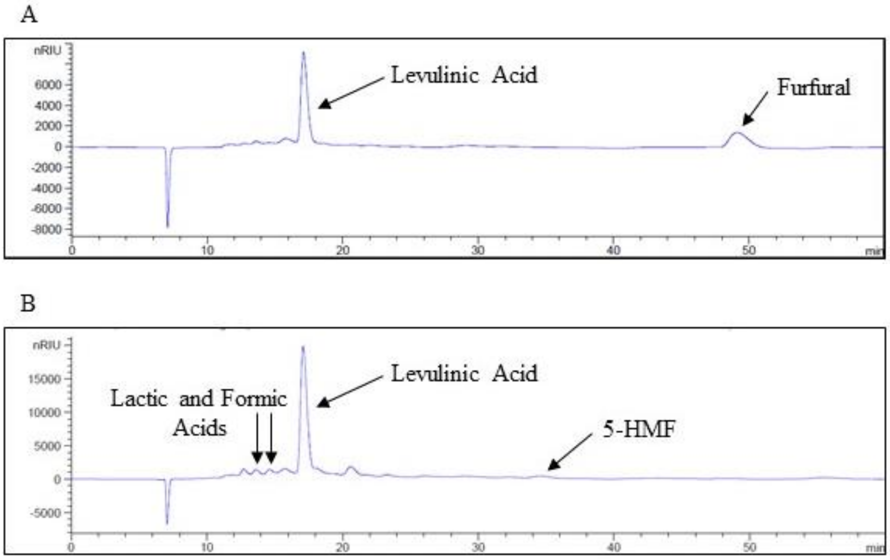

Figure 4. Chromatograms of products obtained from hydrothermal processing of cellulose with acidic ionic liquid: (A) municipal sludge; (B) industrial sludge.

In both chromatograms it is clearly showed that the higher peak (retention time 17.1 min) corresponds to levulinic acid. The other small peaks are the co-products identified in laboratory: furfural, lactic acid, formic acid and 5-hydroxymethylfurfural (5-HMF). Further analysis of the products by LC-MS allowed to identify more substances in the products. Table 6 presents the list of substances detected by LC-MS, the retention time of each compound and molecular mass of positive and negative ion electrospray analysis.

Table 6. Products identified by LC-MS after hydrothermal treatment.

Entry	**Retention Time (min)**	**m/z +**	**m/z –**	**Compound**
1	8.093	117.0188	115.0031	Fumaric acid
2	10.709	135.0293	133.0137	Malic acid
3	11.625	181.0712	N.D.	Mannose
4	11.653	N.D.	179.0556	Glucose
5	13.558	91.0395	89.0239	Lactic acid
6	13.791	119.0344	117.0188	Succinic acid
7	13.924	93.0552	91.0395	Glycerol
8	14.491	97.0290	95.0133	Furfural
9	14.602	175.0243	173.0086	cis-Aconitic acid
10	14.849*	N.D.	59.0133	Acetic acid
11	16.124	111.0421	87.0446	Butyric acid
12	19.473	117.0552	115.0395	Levulinic acid
13	23.863	151.0243	149.0086	Tartaric acid
14	43.633	127.0395	125.0239	5-HMF

N.D. not detected. * Only detected in products obtained from municipal sludge.

All compounds, except acetic acid, were detected in the products obtained from both types of sludge. Acetic acid was only presented in products obtained from municipal sludge. Almost all compounds are short-chain organic acid. These are the expected products of hydrolysis of the cellulose [30]. Apart from organic acids, products of conversion of both sludges contain also glucose, furfural and 5-HMF.

The peak of furfural has appeared in the chromatogram of conversion products of industrial sludge, Figure 4 (a), but not in that of municipal sludge, Figure 4 (b). LC-MS analysis confirmed the peak of furfural in both cases. Furfural comes from the dehydration of xylose. On the other hand, xylose makes up most of the hemicellulose. In Table 4 it could be observed that the hemicellulose content in industrial sludge is double that in municipal sludge. For this reason, the furfural peak is observed in the chromatogram of industrial sludge and not in that of municipal sludge. In the case of 5-HMF, the conversion reactions were carried out at temperatures below 200ºC and therefore large amounts of 5-HMF were not produced. A 5-HMF peak cannot be seen in the chromatogram of conversion products of industrial sludge, Figure 4 (a). However, in the chromatogram of conversion products of the municipal sludge, Figure 4 (b), a very small one appears, as a result of which the 5-HMF was not completely dehydrated to levulinic acid.

On the other hand, Table 5 also presents the weights and the yields of residual solids. Calculation was made based on the weight of biomass used in each reaction. The residual solids were in water insoluble dark-brown solids. The solids after filtration were abundantly washed with water to eliminate the remaining ionic liquid. Then, they were dried and weighted. As it can be seen in the table, depending on the source of cellulose used for the reaction of conversion to levulinic acid, the obtained yield of humins differ. Commercial cellulose (Sigmacell) obtained conversions to humins of ~ 20% except in the case of the experiment carried out for two hours, where the conversion reached was 66%. This increment was caused by insufficient the time to convert cellulose to products. In the case of all the other experiments, purified celluloses from municipal and industrial sludges, or from both sludges used as received, the conversions to humins were higher and quite similar, between 80 and 90%. The yields of residual wastes formed during the reactions with cellulose from sludges was due to the content of ash and proteins in municipal and industrial sludge. After cellulose extraction and purification, a part of ashes and proteins still remained in the cellulose. Subsequently, during the reaction, those organic and inorganic matters pass to the solid residue, at the same time, increasing the yield of humins. Figure 5 shows the FTIR spectra of humins obtained after reaction of purified cellulose from both sludges.

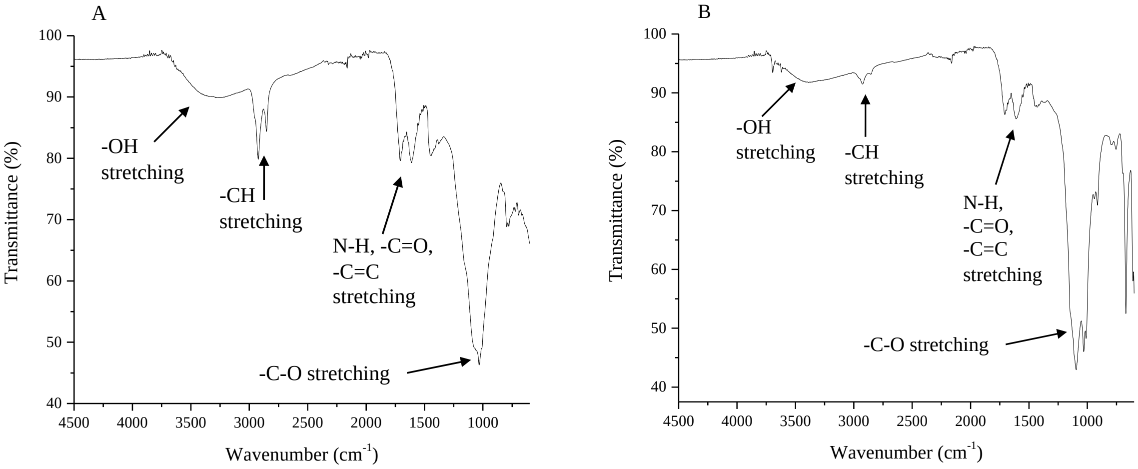

Figure 5. FTIR spectrum of humins obtained from hydrothermal processing of cellulose with acidic ionic liquid: (A) municipal primary sludge; (B) industrial sludge.

In both spectra it can be observed some characteristic absorbances in different frequency regions. The peak at 3400 cm^{-1} is associated with hydroxyl group stretching vibrational bands. The peaks at 2950 cm^{-1} and 1000-1250 cm^{-1} are attributed to -CH and -CO stretching vibrations, respectively. It is also possible to observe strong peaks at 1700 cm^{-1} and 1625 cm^{-1}, corresponding to carbonyl group conjugated to an alkene group. This is because carbohydrates are converted into humins according to the reaction pathway: cellulose ⇨ 5-HMF ⇨ 2,5-dioxo-6-hydroxyhexanal ⇨ humins [14,31]. Although, the peak at 1625 cm^{-1} is also associated with peptide amide groups of proteins. The FTIR spectra confirm that except humins, ashes, proteins and part of unreacted cellulose goes to insoluble solids.

To summarize, the full process of production (extraction, bleaching and catalyzed hydrothermal liquefaction) of levulinic acid from municipal or paper industry has been a success. It is true that the conversions obtained are not high, but it must be taken into account that the entire study is in fact a technological feasibility study. A scale-up of the process can only be carried out when some aspects of the process units will have been solved: (i) optimizing the extraction of cellulose from both sludges through an improved design of the ionic liquid; (ii) improve the cleaning of the cellulose after its extraction, although if more severe methods are used, a greater part of it can be hydrolyzed; (iii) optimize the reaction operations in the conversion of cellulose to levulinic acid, reaction time and temperature, cellulose / ionic liquid / water ratios or improvement in the design of the ionic liquid.

3. Materials and Methods

3.1. Reagents

All chemical reagents used in this work were supplied by Sigma-Aldrich. Tetrakis (hydroxymethyl) phosphonium chloride ($P(CH_2OH)_4]Cl$, hydrated ionic liquid, 80% in water, ref: 404861) was used to recover the cellulose from municipal primary sludge and from industrial sludge. Hydrogen peroxide solution (≥ 30% ref: 95321) and 0.1 N hydrochloric acid solution (ref: 2104) were applied to purify recovered cellulose. 1-methylimidazole (purity ≥ 99%, ref: 336092), 1,4-butanesultone (purity ≥ 99%, ref: B85501) and sulfuric acid (95.0 - 97.0%, ref: 30743) were used to synthesize the Brønsted acidic IL used in the hydrothermal conversion of recovered cellulose to levulinic acid. Methyl isobutyl ketone (MIBK) (purity ≥ 99.5%, ref: 293261) was used to extract levulinic acid from Brønsted acidic ionic liquid after the process of conversion. Pure cellulose Sigmacell (ref: S3504) was used to realize comparative experiments of hydrothermal liquefaction. Glucose (purity ≥ 99.5%, ref: 49139), levulinic acid (98 wt%, ref: L2009), 5-hydroxymethylfurfural (purity ≥ 98.0%, ref: 53407), formic acid (purity ≥ 98%, ref: 33015-M), DL-lactic acid (purity 90%, ref: 69785) and furfural (purity 99%, ref: 185914) were used to prepare calibration curves for high-performance liquid chromatography (HPLC).

3.2. Sludge collection and managing

Cellulose was extracted from two types of sludge: municipal primary sludge and paper industry sludge. The primary sludge (96% water content) was collected after partial gravity thickening of the Wastewater Treatment Plant (WWTP), located in Reus, Spain. The industrial dehydrated sludge (53% water content) was provided by the paper international company Gomà-Camps, S.A., La Riba, Tarragona, Spain. After collection, both types of sludge were stored in a fridge at 4ºC and were used for characterization as received. Prior recovering of cellulose, both sludge were dried in an oven at 105ºC during 48h. Then, they were directly used for the rest of the procedures.

3.3. Sludge characterization

A full characterization of each sludge was realized in triplicate. Determination of total solids (TS), volatile solids (VS), and content of ash, proteins, lipids and carbohydrates were proceeded by conventional methods mentioned in previous studies [22,25].

Total solids (TS) of each sludge were determined by the standard method 2540B [32]. Wet primary sludge and dried paper industry sludge with known quantity (between 0.5 to 2.0 g) were placed in a previously weighted ceramic capsule and then they were put in the oven at 105º for 24h. Total solids were calculated with the formula:

$$\%TS = \frac{(Weight\ dry\ capsule\ plus\ dry\ sample\ g - Weight\ dry\ capsule\ g)}{Weight\ sample\ as\ received\ g}\ x\ 100$$

Volatile solids (VS) and ash content were determined by the standard method 2540E [32]. The weighted dry capsule with dry sample was placed in the muffle at 550ºC for 1h. Ash and volatile solids were calculated with the formulas:

$$\%Ash = \frac{(Weight\ of\ capsule\ and\ sample\ after\ 550\ ℃\ g - Weight\ dry\ capsule\ g)}{(Weight\ dry\ capsule\ plus\ dry\ sample\ g - Weight\ dry\ capsule\ g)}\ x\ 100$$

$$\%VS = 100 - \%Ash$$

Lipids content of each sludge was determined by the standard method 5520E [32]. 20g of sample of each sludge was acidified with 0.3 ml of HCl. Then, 25 g of $MgSO_4 \cdot H_2O$ was added in order to dewater acidified samples of sludge. The samples were mixed and then, they were crushed until they became a fine powder. Then, the samples were transferred into the cellulose Soxhlet extraction thimble and covered with a glass wool. The extraction was done in a Soxhlet extractor using hexane as a solvent at a velocity of 20 cycles/hour for 4 h. After all, the hexane was evaporated through a rotary evaporation. The lipids were kept in a desiccator overnight to remove remained traces of hexane and weighed the next day. The flasks were measured before and after the experiment. Mass of lipids was calculated by weighing and with the formula:

$$\%Lipids = \frac{(Weight\ Lipids + flask\ g - Weight\ of\ flask\ g)}{Weight\ of\ TS\ g} x100$$

Proteins content of each sludge was determined by the Lowry method using Folin phenol reagent [33]. Firstly, the calibration curve of 0.5 g Bovine serum albumin/L patron was prepared. Then, diluted samples of each sludge were heated with 2M solution of NaOH in order to dissolve proteins. The absorbance of patron as well as sludge samples were measured at 750 nm. The amount of proteins was calculated by the colorimetric method.

Carbohydrates determination was carried out based on phenol sulphuric method of Dubois [34]. Calibration curve of 0.1 g glucose/L was prepared as a patron. Diluted samples of each sludge were mixed with phenol solution and then with concentrated sulphuric acid. The absorbance of patron as well as sludge samples were measured at 480 nm. The amount of carbohydrates was calculated by the colorimetric method.

3.4. Recovery of cellulose from sludge

The recovery of cellulose from both types of sludge was carried out using the method described in previous studies [22-24], with small modifications. Briefly, instead of wet sludge, dry sludge (DS) was used. This was necessary to prepare a big batch of recovered cellulose. The use of wet sludge (96% or 53% water content) would require working with too big volumes of sludge and ionic liquid. Dried sludge was placed in a round bottom flask and Tetrakis IL was added in ratio $1g_{DS}$:10 mL_{IL}. The mixture was stirred and heated to 100ºC with an oil bath maintained 24 hours. After this time, the oil bath was removed and the flask cooled down to ambient temperature. At that point, 10 mL of water for each gram of dry sludge were added to the solubilized cellulose. The rest of the procedure was the same as in the previous studies. After reaction, IL was recovered by water evaporation in a rotary evaporator at 80ºC and 300 mbar. Then, IL was ready to be reused for other successive recovering.

3.5. Characterization of recovered cellulose

The cellulose was analyzed to determine the volatile solid and ash content according to standard method 2540E [32]. The amount of proteins was calculated as a difference between proteins in sludge as received and proteins determined in ionic liquid phase by Lowry method [33]. Cellulose, hemicellulose and lignin were analyzed by high performance liquid chromatography (HPLC) after strong hydrolysis following method presented elsewhere [24].

3.6. Bleaching of recovered cellulose

Bleaching method was carried out according to literature [26]. Dried recovered cellulose from both sludge was placed in a round bottom flask. Then, 250 mL of freshly prepared 8% H_2O_2 (v/v) was added. The mixture was kept under agitation at room temperature during 24 h. After that, H_2O_2 and solids were separated by centrifugation (8000 rpm, 5 min). H_2O_2 was kept in order to determine the content of proteins by Lowry method [33] and, the ash content by the conventional method 2540E [32]. On the other side, the solid was washed with distilled water and centrifuged (8000 rpm, 5min). Then, the solid was hydrolyzed with 250 mL of 0.1N HCl in an ultrasonic bath (Bandelin Electronic DT 514H) at a temperature of 60 ± 1ºC for 5 h. Afterward, the liquid was removed by centrifugation (8000 rpm, 5min) and the solid was washed several times with distilled water until neutralization of the washing liquid of the solid. At the end, the solid was dried overnight in the oven at 105ºC and analyzed for ash, proteins, cellulose, hemicellulose and lignin content as it was described before. Additionally, purified cellulose was characterized by Fourier Transform Infrared (FTIR) spectroscopy. The samples, without any further preparation, were scanned using a Fourier Jasco FT/IR-600 Plus spectrometer with a diamond golden gate ATR reflectance cell.

3.7. Synthesis of the Brønsted acidic ionic liquid

The Brønsted acidic ionic liquid 4-(3-methylimidazolium) butanesulfonic acid hydrogenosulfate [mimC$_4$SO$_3$H][HSO$_4$] was prepared according to literature [25]. Briefly, equimolar quantities of 1-methylimidazole and 1,4-butanesultone were added to a round bottom flask fitted with a condenser and stirred at 80ºC for 10h under nitrogen purge. Then, the obtained solid was washed with toluene and diethyl ether and, dried under vacuum overnight. A stoichiometric amount of acid H_2SO_4 was added dropwise to the white solid and stirred overnight at 60ºC under reflux condenser and nitrogen purge. The obtained viscous IL was dried under vacuum and characterized by 1H NMR spectroscopy (Varian NMR System 400).

The IL was characterized by ^{1}H NMR spectroscopy, to confirm its structure. ^{1}H NMR spectrum (400 MHz, d^4-methanol), d (ppm): 1.79 (p, 2H, CH_2, J=7.2), 2.05 (p, 2H, CH_2, J=7.6), 2.9 (t, 2H, CH_2-SO3H, J=7.6), 3.94 (s, 3H, N-CH_3), 4.27 (t, 2H, N-CH_2, J=7.2), 7.59 (t, 1H, *CH*, J=1.2), 7.66 (t, 1H, *CH*, J=1.6), 8.96 (s, 1H, N-*CH*-N).

3.8. Conversion of purified cellulose to levulinic acid

3.8.1. Reaction experimental procedure

The procedure of conversion of cellulose to levulinic acid, presented in Figure 1, is based on a recent work [3]. Different amounts of purified cellulose (2.2 to 16.0 g), Brønsted acidic ionic liquid (26.0 to 36.0 g) and 180 mL of distilled water were utilized. The substances were introduced in a 1 L stainless steel autoclave (Autoclave Engineers model EZE Seal) with heating shell and MagneDrive® stirrer (magnetically coupled, packless rotary impeller system). The amounts of purified cellulose and IL were chosen based on the best conditions described in the work of Ren et al., 2015 [3]. Two last experiments were carried out with wet primary sludge as received (96% water content) and paper industry sludge as received (53% water content). These two experiments, considered as blank assays, allow the comparison of performances with the other experiments realized with bleached sludge. After closing the reactor, the temperature was settled up to 170ºC. The reactor took ~30 min to reach the desired temperature. The pressure in the reactor at that temperature was over 7 bars and was constant during all the reaction time. Two different times of reaction were used after reaching the process temperature: 2 and 5 h. After the reaction, the heating shell was removed and the system was cooled in cold water. The cooling time was ~35 min. After cooling, the reactor went back to atmospheric pressure.

3.8.2. Cleaning of the reactor and separation of products

After opening of the reactor, the content was poured into a beaker and the reactor interior itself was cleaned with deionized water to recover all products: a black mixture of insoluble solids and liquid. Then, the dark brown insoluble solids, also called humins [3,35], were separated from the liquid phase by filtration with a Büchner funnel. The solids were washed with water several times. Then, they were oven dried at 105ºC for 24h and weighed. The yield of insoluble solids (%) was calculated with the initial weight of biomass used in the process. Furthermore, the solids were characterized by FTIR spectroscopy.

The liquid phase, mixture of water, IL and products, was left overnight in the oven at 105ºC to evaporate the water. Then, the organic products were solubilized with methyl isobutyl ketone (MIBK) and separated from the ionic liquid by centrifugation at 8000 rpm for 5 min. The formation of two phases, MIBK and products (upper layer) and ionic liquid (down layer), allowed an easily separation of products from the ionic liquid. The upper layer was gathered and then, MIBK was evaporated at the rotary evaporator at 85ºC and 250 mbar. At that point, products were dried under vacuum and weighted. Products were characterized by HPLC (Agilent Technologies 1100 Series HPLC System). For this, they were dissolved in water and analyzed by HPLC equipped with a Refractive Index Detector (RID) and a Hi-Plex H column (300 x 7.7 mm), filled by a robust sulfonated cross-linked styrene-divinylbenzene gel in hydrogen form. Chromatograph conditions were as follow: injection volume 20.0 µL, mobile phase 0.005M H_2SO_4, flow rate 0.7 ml/min, column temperature 60ºC, RID temperature 55ºC. The concentration of each compound was determined using calibration curves obtained with standard solutions with known concentrations. The yield of levulinic acid (%) was calculated from the initial weight of cellulose used in the reaction. Additionally, products were analyzed by LC – MS with an Agilent 1200 liquid chromatograph, coupled to a 6210 Time of Flight (TOF) mass spectrometer (Agilent Technologies, Waldbronn, Germany) and with an electrospray ionization (ESI) interface. Experiments were performed using a Hi-Plex H column (300 × 7.7 mm) provided by Agilent Technologies under isocratic conditions (H_2O + 0.1 % HCOOH) and a constant flow of 0.7 mL/min. The HPLC eluate was directly pumped into ESI interface without flow splitting. The ESI conditions were as follow: gas temperature 300 ˚C, drying gas 12 L/min, nebulizer 40 psi, fragmentor 120 V, capillary voltage (3000 V), mass range 50 - 1200.

4. Conclusions

The process of production of levulinic acid from municipal or paper industry sludges has allowed to obtain very interesting results: (i) the Tetrakis (hydroxymethyl) phosphonium chloride ionic liquid allows the extraction of all the cellulose in both sludges, however the precipitated cellulose still contains proteins and ashes; (ii) the bleaching process by hydrogen peroxide and hydrochloric acid does not work as well as expected, after the process the cellulose continues to have impurities that will be annoying in the reaction; (iii) catalyzed hydrothermal liquefaction of cellulose is a process that makes it possible to produce levulinic acid, but the operating conditions have to be optimized; (iv) it is possible to carry out an integrated process for the production of levulinic acid from residual biomass, in this case sludge from municipal or industrial treatment plants. Finally, it should be noted that the obtained conversions to levulinic acid are not high, up to 31% with industrial sludge and up to 25% with municipal sludge. But it must be emphasized that the entire project is a technological feasibility study. The use of waste from wastewater treatment plants can lead to an increase in their income when it is well known that the cost of disposal their wastes is the biggest part of their operational costs.

Author Contributions: Conceptualization, Christophe Bengoa; Data curation, Jaume Giralt and Christophe Bengoa; Funding acquisition, Jaume Giralt and Christophe Bengoa;

Investigation, Katarzyna Glińska and Clara Lerigoleur; Methodology, Katarzyna Glińska, Esther Torrens and Christophe Bengoa; Project administration, Jaume Giralt; Resources, Jaume Giralt and Christophe Bengoa; Supervision, Christophe Bengoa; Validation, Esther Torrens and Christophe Bengoa; Visualization, Katarzyna Glińska and Christophe Bengoa; Writing – original draft, Katarzyna Glińska and Clara Lerigoleur; Writing – review & editing, Katarzyna Glińska, Esther Torrens and Christophe Bengoa.

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Chapter 7

Conclusions and future work

1. Conclusions

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

- Industrial paper mill wastewater dried sludge is a mixture of carbohydrates and ashes while the primary sewage sludge is more complex mixture of organic compounds (lipids, proteins and carbohydrates) and inorganic ashes. Both types of sludge are potential source of carbohydrates, mainly of cellulose.
- The ionic liquid tetrakis hydroxymethyl phosphonium chloride $[P(CH_2OH)_4]Cl$ has shown potential for the recovery of the cellulose from both types of sludge. The amount of carbohydrates measured by the conventional method in sludge and the amount of cellulose recovered using ionic liquid method are comparable. Also, that cheap and commercially available ionic liquid has ability to dissolve part of ashes and proteins.
- The ionic liquid 1-butyl-3-methyl imidazolium methyl sulphate presents great recoveries of cellulose from rehydrated dried sludge provided by an industrial paper company. The comparison of both ionic liquids shows that tetrakis allows a less content of ashes in the precipitated phase while imidazolium ionic liquid lets a minor quantity of the proteins in the precipitated phase.
- The optimization study about the recovery of cellulose from primary sludge using tetrakis ionic liquid was done. The experimental design was not able to optimize the operative conditions of the process of recovery of carbohydrates. Experiments realised at 60ºC for 13 h and with sludge:IL ratio 1g $_{\text{TS of SLUDGE}}$: 12 mL gave very good results, allowing the recovery of high quantities of cellulose, the best removal of ashes but maintaining high quantities of proteins in the precipitate.
- Residual biomass as corn stover is also a potential source of carbohydrates. Synthesised ionic liquids are able to break cell walls and extract cellulose from corn stover. Microemulsion-forming imidazolium ionic liquids can be considered to be easily recovered ILs.
- The Brønsted acidic ionic liquid shows a good catalytic performance for the conversion of cellulose extracted from primary sewage sludge and paper sludge into a value-added molecule.

2. Future work

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

- Further investigation of cellulose bleaching is required in order to decrease the amount of ashes and proteins in extracted cellulose.
- A topic worthy of development in the future, it to carry out an optimisation study about the conversion of cellulose to value-added molecules in order to increase the yields of the products.
- As a recommendation for cellulose extraction from municipal sewage sludge, a scaled process should be done to perform viability and economic feasibility evaluations.
- As demonstrated in this thesis, the ionic liquid used to recover cellulose from residual biomass can be recovered. However, in this work, it was performed as a separate process, it would be interesting to design it as a continuous process.
- Another recommendation for cellulose extraction from residual biomass would be the design of improved ionic liquids able to solubilize the cellulose and remove all ashes and proteins.

Appendix

Thesis outcomes

Publications

Authors: Katarzyna Glińska, Mohammed Aqlan, Jaume Giralt, Esther Torrens, Daniel Montané, Frank Stüber, Azael Fabregat, Agusti Fortuny, Josep Font, Magdalena Olkiewicz, Christophe Bengoa

Title: Separation of cellulose from industrial paper mill wastewater dried sludge using a commercial and cheap ionic liquid

Journal: Water Science & Technology

Volume: 79.10 **Pages:** 1897-1904 **Year:** 2019

Authors: Katarzyna Glińska, Muhammad Solehin Bin Ismail, Jordi Goma-Camps, Pau Valencia, Frank Stüber, Jaume Giralt, Azael Fabregat, Esther Torrens, Magdalena Olkiewicz, Christophe Bengoa

Title: Recovery and characterization of cellulose from industrial paper mill sludge using tetrakis and imidazolium based ionic liquids

Journal: Industrial Crops & Products

Volume: 139 **Pages:** 111556 **Year:** 2019

Authors: Katarzyna Glińska, Frank Stüber, Azael Fabregat, Jaume Giralt, Josep Font, Josep Maria Mateo-Sanz, Esther Torrens, Christophe Bengoa

Title: Moving municipal WWTP towards circular economy: Cellulose recovery from primary sludge with ionic liquid

Journal: Resources, Conservation & Recycling

Volume: 154 **Pages:** 104626 **Year:** 2020

Authors: Katarzyna Glińska, Jaume Giralt, Esther Torrens, Natalia Plechkova, Christophe Bengoa

Title: Extraction of cellulose from corn stover using designed ionic liquids with improved reusing capabilities

Journal: Process Safety and Environmental Protection

Volume: 147 **Pages:** 181-191 **Year:** 2021

Authors: Katarzyna Glińska, Clara Lerigoleur, Azael Fabregat, Josep Font, Agusti Fortuny, Jaume Giralt, Frank Stüber, Esther Torrens, Christophe Bengoa

Title: Valorisation of cellulose recovered from WWTP sludge to added value levulinic acid with a Brønsted acidic ionic liquid

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Conference contributions

K. Glińska, A. Fabregat, J. Giralt, E. Torrens, F. Stüber, A. Fortuny, D. Montané, J. Font, M. Olkiewicz, C. Bengoa. Recuperación de material celulósico de lodos primarios municipales usando líquidos iónicos. Oral presentation at the VII Jornada sobre gestión y tratamiento de LODOS de EDAR, 22 November 2017, Barcelona, Spain.

K. Glińska, M. Aqlan, E. Torrens, F. Stüber, A. Fabregat, A. Fortuny, J. Giralt, J. Font, D. Montané, M. Olkiewicz, C. Bengoa. Extraction of cellulose from industrial paper mill wastewater sludge using ionic liquid. Oral presentation at International Conference of Sludge Management in Circular Economy “SMICE”, 23-25 May 2018, Rome, Italy.

K. Glińska, A. Fabregat, J. Giralt, E. Torrens, F. Stüber, A. Fortuny, D. Montané, J. Font, M. Olkiewicz, C. Bengoa. Cellulosic material recovery from municipal primary sludge using ionic liquids. Oral presentation at International Conference of Sludge Management in Circular Economy “SMICE”, 23-25 May 2018, Rome, Italy.

M. Solehin Bin Ismail, K. Glińska, E. Torrens, M. Olkiewicz, C. Bengoa. Extraction of cellulose from industrial paper mill wastewater sludge using ionic liquid. Poster presentation at the 4th Iberoamerican Congress on Biorefineries, 24-26 October 2018, Jaen, Spain.

K. Glińska, M. Solehin Bin Ismail, E. Torrens, M. Olkiewicz, C. Bengoa. Extraction of cellulose from industrial paper mill wastewater sludge using ionic liquids. Poster presentation at 16th Doctoral Day, Universitat Rovira I Virgili, 22 May 2019, Tarragona, Spain.

K. Glińska, M. Solehin Bin Ismail, J. Goma-Camps, P. Valencia, J. Giralt, E. Torrens, F. Stüber, A. Fabregat, M. Olkiewicz, C. Bengoa. Recuperación y caracterización de celulosa a partir de fango industrial de papel usando líquidos iónicos. Oral presentation at the Seminario Técnico “Tratamiento de aguas industriales”, 4-5 July 2019, Oviedo, Spain.

K. Glińska, N. Plechkova, J. Font, E. Torrens, C. Bengoa. Cellulose extraction from corn stover using synthesised ionic liquids. Oral presentation at the 4th International Conference on Ionic Liquids in Separation and Purification Technology (ILSEPT), 8-11 September 2019, Sitges, Spain.

Curriculum Vitae

Katarzyna Glińska was born in Ełk, Poland, in 1990. She obtained her BSc degree in Chemistry, specialising in Cosmetic Chemistry, in 2012 at the Chemistry Faculty of Adam Mickiewicz University in Poznań, Poland. Then, in 2014, she pursued a MSc degree specialising in Material Chemistry at the same university. During her master's degree she took part in Erasmus Lifelong Learning Programme, she spent six months in Chemical Engineering Department of Universitat Rovira i Virgili in Tarragona, Spain. Then, six months more, she spent on doing the research for her master thesis in Analytical and Organic Chemistry Department of Universistat Rovira i Virgili. In 2016 she was awarded a Martí- Franquès Research Fellowship Programme to undertake the Ph.D. research at the Department of Chemical Engineering of Universitat Rovira i Virgili 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. She was synthesising and characterizing ionic liquids able to extract cellulose from residue biomass.

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