Journal of Chemical Technology and Biotechnology

Enzymatic hydrolysis from carbohydrates of barley straw pretreated by ionic liquids

Journal:	*Journal of Chemical Technology & Biotechnology*
Manuscript ID:	JCTB-12-0530.R1
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Sáez, Felicia; CIEMAT, Energy Department Ballesteros, Mercedes; CIEMAT, Energy Department Ballesteros, Ignacio; CIEMAT, Energy Department Manzanares, Paloma; CIEMAT, Renewable Energies Oliva, Jose; CIEMAT, Renewable Energies Negro, María José; CIEMAT, Renewable Energies Division-Biomass Unit
Key Words:	Bioconversion, Enzymes, Pretreatment, Biomass

Enzymatic hydrolysis from carbohydrates of barley straw pretreated by ionic liquids

F. Sáez, M. Ballesteros, I. Ballesteros, P. Manzanares, J.M. Oliva, M.J. Negro*

Biofuels Unit, Renewable Energy Department-CIEMAT, 28040Madrid, SPAIN

e-mail: mariajose.negro@ciemat.es

ABSTRACT

BACKGROUND: Lignocellulosic biomass offers many potential advantages in comparison with the traditionally used sugars or starchy biomass since it's vastly available and it does not compete with food and feed production. The abundance and high carbohydrates content of barley straw make it a good candidate for bioethanol production in Europe. Since biomass must be pretreated before enzymatic hydrolysis to improve the digestibility of both the cellulose and the hemicellulose biomass, the use of ionic liquids (IL) has been proposed as an environmental friendly pretreatment of biomass.

RESULTS: Different pretreatment conditions were investigated to determine the effects of the experimental conditions (temperature and time) on the enzymatic digestibility of pretreated material. The pretreatment of barley straw with 1-ethyl-3-methyl imidazolium acetate treatment resulted in up to a 9-fold increase in the cellulose conversion and a 13-fold increase in the xylan conversion when compared with the untreated barley straw.

CONCLUSION: Ionic liquid pretreatment of barley straw at 110 ºC for 30 minutes, followed by enzymatic hydrolysis, leads to a sugar yield of 53.5 g/100 g raw material. It's then ready available for conversion into ethanol and is equivalent to more than 86%

from potential sugars. The increase in saccharification was possible due to the rupture in lignin-hemicellulose linkages with treatment of 1-ethyl-3-methyl imidazolium acetate.

Keywords: Lignocellulose, cellulose digestibility, biomass pretreatment, ionic liquid, barley straw.

INTRODUCTION

Ethanol production from biomass has gained considerable interest in order to provide energy security and reduce greenhouse-gas emissions. Lignocellulosic biomass offers many potential advantages in comparison with the traditionally used sugary or starchy biomass for its large quantity and not competing with food and feed production. Furthermore, lignocellulosic ethanol has shown to involve up to 85 % net reduction in greenhouse-gas emissions[1]. Among lignocellulosic substrates, barley straw is a good candidate for bioethanol production in Europe due to its high availability and high carbohydrates content[2].

In the lignocellulosic biomass to ethanol process based on the use of hydrolytic enzymes, biomass must be pretreated before enzymatic hydrolysis to improve the digestibility of both cellulose and hemicellulose fractions. The pretreatment consists of breaking the lignocellulose matrix to expose the carbohydrates for enzymatic reaction. Among pretreatment methods, the use of ionic liquid has been proposed as an environmental friendly pretreatment of biomass[3,4].

The use of ionic liquids (ILs) as solvents for pretreatment of cellulosic biomass has received attention during the last decade. ILs are capable of breaking down the extensive hydrogen-bonding network in the polysaccharides and promote its solubilization. ILs are generally defined as salts that melt at or below 100 ºC, affording

liquids exclusively composed of ions[5]. These salts show unique properties including an almost negligible vapour pressure and high solvatation capacity, which make them ideal solvents for a range of applications[6]. Their solvent properties can be varied by adjusting the anion and the alkyl constituents of the cation[7] .

Although most available data showing the effectiveness of ILs have been developed using pure crystalline cellulose, recent studies have demonstrated that ionic ILs can be used to pretreat lignocellulosic biomass such as sugarcane bagasse[8,9] , wheat straw[10]or wood[11] . Several imidazolium-based ILs were originally reported as good methods to dissolve large amounts of cellulose[12]. Acetate-based ILs are considered an attractive choice for processing cellulosic biomass in a efficient and environmentally friendly way, as they dissolve large amounts of cellulose under very mild conditions and can be nearly 100% recovered[7] .

The 1-ethyl-3-methyl imidazolium acetate is currently regarded as one of the most effective pretreatment solvents, as its application partly dissolves lignocelluloses while also achieving substrate delignification and the decrystallization of cellulose[11,13]. Some authors have reported that IL-pretreatment of switchgrass significantly improves the enzymatic saccharification of both cellulose (96% glucose yield in 24 h) and xylan (63% xylose yield in 24 h)[14]. This improvement is attributed to the reduction in cellulose crystallinity and the delignification effect during dissolution-regeneration steps. However, scarce information about ILs pretreatment on barley straw has been found.

For the large-scale application of ILs, development of energy-efficient recycling methods for ILs is a prerequisite and should be investigated in detail15[15]. Toxicity to enzymes and fermentative microorganisms must also be studied before ILs can be considered a real option for biomass pretreatment[16]. Despite of these current limitations, advanced research such as potential synthesis of ILs from carbohydrates,

may play a role in reducing their cost. Development of ILs pretreatment could offer a great potential for future lignocellulose biorefinery processes.

In this work, the enzymatic hydrolysis of barley straw pretreated with ionic liquids was studied for the first time. Different pretreatment conditions were investigated to determine the effects of the experimental conditions on the sugar yields by enzymatic hydrolysis.

MATERIAL AND METHODS

Raw Material

Barley straw (*Hordeum vulgare*, 6-7 % moisture), supplied by CEDER (Spain) was used as raw material. The analysis showed the following composition (% dry weight): glucan 34.9 ± 0.8, xylan 20.8 ± 0.6; arabinan 2.5 ± 0.01, galactan 0.8 ± 0.01 and acid insoluble lignin 18.1 ± 0.8. Its composition was determined by total acid hydrolysis using the standard Laboratory Analytical Procedures for biomass analysis provided by the National Renewable Energy Laboratory (NREL) (Colorado, USA)[17]

Pretreatment

A barley straw sample (500 mg) was dissolved in 9.5 g of hot 1-ethyl-3-methyl imidazolium acetate [EMIM][OAc] (manufactured by BASF and purchased from Sigma-Aldrich). Assay temperature ranged between 90 and 130 ºC and residence time between 30 and 60 minutes. Pretreatment was performed in a glass vessel into a temperature-controlled oil bath with magnetic stirring. The reaction was stopped by adding the same amount of deionized water (anti-solvent). After the addition of water, the precipitated material was separated by filtration. The liquid was analyzed for momomeric sugars and

degradation compounds by HPLC and the solid fraction was washout thoroughly with water and was characterized as glucan, hemicellulose, and lignin, as described in analytical methods.

Enzymatic hydrolysis.

The solid fraction obtained after pretreatment was used as substrate for enzymatic hydrolysis. Enzymatic microassays[18] were carried out in 2 mL eppendorf tubes. Experiments were performed at 5% (w/v) dry pretreated substrate loading, at 50 ºC for 72 h in a microplate incubator (ThermoStar, 3 mm shaken amplitude) and 800 rpm using citrate buffer 0.05 mol/L at pH 5. Cellulose-hydrolyzing enzymes, Novozyme 50013 with an activity of 65 filter paper units (FPU)/g, and Novozyme 50010 with a β-glucosidase activity of 590 IU/g, were used in all experiments. Enzymes were kindly provided by Novozymes A/S (Denmark). Enzyme loading of 15 FPU/g of dry pretreated substrate of cellulase and 15 IU/g pretreated substrate of glucosidase was used. After 72 h enzymatic hydrolysis, glucose and xylose content was analyzed by HPLC. Enzymatic hydrolysis yields (EH_g and EH_x) were calculated as the ratio of glucose and /or xylose release divided by potential glucose and /or xylose content in solid fraction.

Analytical methods.

The chemical composition of the raw material and the solid fraction obtained after pretreatment was determined according to NREL method[17]. Sugars concentration was determined by high performance liquid chromatography (HPLC) in a Waters 2695 liquid chromatograph with refractive index detector. A CARBOSep CHO-682 LEAD column (Transgenomic, Omaha, NE) operating at 75 ºC with Milli-Q water (Millipore) as mobile-phase (0.5 mL /min) was used.

Phenolic compounds were analyzed by HPLC (Agilent, Waldbronn, Germany) employing an Aminex HPX-87H column (Bio-Rad Labs, Hercules, CA) at 65 ºC. The mobil phase contained 89% (5 mM H_2SO_4) and 11% acetonitrile at flow rate of 0.7 mL/min. A 1050A Photodiode-Array detector (Agilent, Walsbronn, Germany) was employed for detection.

RESULTS AND DISCUSSION

Effect of pretreatment on the composition of regenerated biomass

The 1-n-ethyl-3-methyl-imidazolium acetate was selected as ionic liquid (IL) for biomass pretreatment, due to its low melting point temperature, and it is liquid at room temperature. Moreover, it has low viscosity and it is easy to handle. The imidazolium group is substituted with relatively short alkyl chains. According to the bibliography, ionic liquid with short-chain groups have been found to be less toxic than long-chain[19]. On the other hand, the acetate ion is less corrosive than the IL's halide anions, which have also been described as effective in cellulose dissolution.

It is worth mentioning that the IL-pretreated biomass suspension showed a dark brown colour, soon after the onset of the reaction, indicating that IL's has a good ability to extract lignin from barley straw. Moreover, a complete dissolution of the barley straw was observed in all pretreatment conditions, except for pretreatment carried out at 90 ºC for 30 minutes. The colour change of the [EMIM][OAc] following pretreatment was also reported for sugarcane bagasse[13]

Table 1 shows the yield of regenerated biomass (solid obtained after solubilisation and precipitation, divided by original oven-dried weight) and composition of insoluble solids obtained after pretreatment (regenerated solid). Yield of regenerated biomass was in the range of 74.7-80.3 %. Similar regenerated biomass yields values were obtained in

other herbaceous materials such as switchgrass, while lower to those obtained in poplar using the same ionic liquid[20].

Glucan (values ranging from 35 to 45.6 %), is the main component of insoluble solids (regenerated solid). Hemicellulose content in the regenerated solid ranges from 21.8 to 28.6%. Acid insoluble lignin (AIL) content of regenerated solid, varied 16.2-18.5%. On the other hand, when these values are referred to untreated material, the glucan content varies from to 26.2 to 34.1 %, which means a recovery of glucan in the range 75-97.4 % in the solid fraction. Hemicellulose recovery in the solid fraction varies from 77.3 to 94.1 %. High values of hemicellulose-sugars recovery in the pretreated solid would be interesting to increase the total fermentable sugars production. Our results are in accordance with those obtained in pretreated wood flour with the same IL for 90 min at 130ºC removed 16% and 26% of cellulose and hemicellulose using the same IL[11]. Similarly the pretreatment of swithgrass with [EMIM][OAc] at 160ºC resulted in an 80% of the original glucan recovery in the regenerated solid[21].

In the liquid fraction (IL plus water) low recovery of monomeric sugars (Table 2), less than 1 g/100 g raw materials, was obtained. The hydrolysis of cellulose (glucan) and xylan to monomeric sugars in the liquid fraction (IL and water) was negligible. These results agree with those obtained by other authors [22,23]. The liquid fraction was also analyzed for furans content, and neither HMF nor furfural were detected, which is consistent with low glucose and xylose found in the soluble fraction. In contrast, phenolic compounds were detected in the liquid fraction. Wide range of phenolic compounds derived from lignin decomposition is generated during pretreatment as shown in Table 2 where the recovery of phenols in the liquid obtained after IL treatment and precipitation with water (anti-solvent) is depicted. Identified phenols are monomers with an aliphatic substituent with different functional groups: aldehydes, ketones or

acids. Phenols as derivatives of cinnamic acids such as acid p-coumaric are found in concentration of 6-13 mg/L (that are equivalent to 23 to 50 mg/100 g raw material). The concentration and type of phenolic compounds are highly dependent on the raw material since lignin content and chemical structure differs among the different lignocellulosic materials. It is worth noticing that vanillin concentration, formed by degradation of guaiacyl propane (G) units of lignin, is significantly higher than syringaldehyde, produced by degradation of syringylpropane (S) units of lignin. This fact is consistent with the G/S ratio in herbaceous biomass[24].

Enzymatic saccharification

The main objective of pretreatment is to alter the structure of the fibres in order to increase the accessibility of enzymes to cellulose. So, the effect of temperature and time on the enzymatic digestibility of the solid fraction obtained after IL pretreatment was evaluated and results are shown in Fig 1. Enzymatic hydrolysis yield depends on the pretreatment temperature and in general, yield increases as the temperature rises. As a general trend it was observed that shorter time pretreatment increases the glucose production in comparison with longer pretreatment time at 110 ºCand 130 ºC. However, when pretreatment was carried out at 90 ºC, the production of glucose increased over time, which could be related to the fact that 30 minutes and pretreatment at 90 ºC results in an incomplete biomass dissolution. The highest glucose production by enzymatic hydrolysis (23.5 g/L) was obtained at 130 ºC for 30 min pretreatment, whereas the highest xylose concentration (11.6 g/L) was obtained at 110 ° C and 30 min. Glucose and xylose yield (EH_g and EH_x) from glucan and xylan, respectively, were reported as a percentage of theoretical yields of monomeric sugars based on the glucan and xylan analysis of pretreated substrates. Low enzymatic hydrolysis yield were obtained at 90ºC 68.0 % cellulose yield and 48.8 % xylan yield. However at higher temperature,

enzymatic hydrolysis yields over 90 % were obtained. The maximum hydrolysis yield at 72 h (100 %) was found in the solid fiber obtained after ILs pretreatment at 110 ºC and 60 minutes. The untreated raw material showed an average value of 11 % theoretical glucose yield in tests performed in parallel to pretreated substrates (data not shown). The Il's pretreatment improved yields of both glucose and xylose sugars. The pretreatment of barley straw with [EMIM][OAc] treatment resulted in up to a 9-fold increase in the cellulose conversion and a 13-fold in the xylan conversion when compared with the untreated barley straw. In other pretreatments the presence of hemicellulose and lignin has been reported to negatively affect the enzymatic hydrolysis; this is not the case of IL, given the high yields of hydrolysis despite considerable amount of xylan in pre-treated substrate (Table 1). Probably it is because of the particular rupture in lignin-hemicellulose linkages with treatment with ionic liquids, which together with the decline of the crystallinity of cellulose[13,25] results in highly hydrolysable substrates by enzymes.

The principal hemicelluloses in herbaceous crops are arabinoxylan and the ferulic acid is covalently linked to arbinoxylans. The increase in yield of sugars in the enzymatic hydrolysis step after IL pretreatment can be attributed the hemicellulose-lignin cross-linkage disruption. Partial delignification and probable lignin redistribution coupled with decrease crystallinity would contribute to enhancement of glucose and xylose yields in the IL pretreated substrates[13] .

Ferulic acid is linked by its phenolic group via ether bond to lignin and also principally linked by its carboxyl group via ester bond to lignin and/or hemicellulose[26]. In Gramineae, these alkali-labile ester linkages involving arabinose predominate over alkali-stable. Hydroxycinnamic acids, such as p-coumaric and ferulic acids, appeared to be strongly linked to lignin molecules, in which p-coumaric acid is bonded to lignin by ester-linkage. Ester linkages are completely broken with alkalis and the ether linkages

were hydrolyzed by acidolysis to cleave to phenolic acids in the lignin. During IL pretreatment the ester linkages could be broken due to the basic properties of IL used in this work, and as consequence the rest of ferulic acid may be still attached to the lignin, so contributing to enhancement of enzyme access to cellulose. This fact could explain that ferulic acid is not found in the liquid fraction after pretreatment as shown in Table 2. While in cereal straw large amount p-coumaric acid was ester-linked to cell wall components, mainly to lignin, these alkali-labile ester linkages could break down during pretreatment and it could explain the presence of p-coumaric in the liquid fraction after IL pretreatment.

In order to optimize the overall process, attention must be paid to several partial objectives, e.g. both cellulose and hemicellulose recovery in the solid residue in the pre-treatment step, and hydrolysis yield in the enzymatic step, some of which may interact with each other. A great cellulose recovery does not guarantee a fine enzymatic hydrolysis. Overall sugar yield, calculated in relation to the raw material for the two phases: pretreatment and enzymatic hydrolysis, is the major indicator of the potential amount of sugars that could be used for ethanol production. Overall sugar yields were evaluated and results are shown in Table 3

At the best conditions (IL pretreatment at 110 ºC for 30 min) an overall yield of glucose of 34.8 g/100 g barley straw and 18.7 g xylose/100 g barley straw was obtained. This figures are highest than those obtained in barley straw pretreated by steam explosion[27].

CONCLUSION

IL pretreatment of barley straw at 110 ºC for 30 minutes followed by enzymatic hydrolysis leads to a sugar yield of 53.5 g/100 g raw material, readily available for conversion into ethanol, equivalent to more than 86 % from potential sugars. The

increase in saccharification was possible caused by the rupture in lignin-hemicellulose linkages with treatment with [EMIM][OAc].

Results obtained in the present study are very promising and will be following up by examining the ethanol production from pretreated materials.

REFERENCES

1 Banerjee S, Mudliar S, Sen R, Giri B, Satpute D, Chakrabarti T and Pandey RA. Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. *Biofuel Bioprod Bioref* **4**:77–93 (2010).

2 Kim S and Dale BE. Global potential bioethanol production from wasted crops and crop residues. *Biomass Bioenerg* **26**:361-375 (2004).

3 Xi H, King A, Kilpelainen I, Granstrom M, and Argyropoulos DS, Thorough chemical modification of wood-based lignocellulosic materials in ionic liquids. *Biomacromolecules* **8** (12): 3740-3748 (2007).

4 Zhu, S, Use of ionic liquids for efficient utilization of lignocellulosic materials. *J Chem Technol Biotechnol* **83:777**-779 (2008)

5 Tadesse, H and Luque R, Advances on biomass pretreatment using ionic liquids: an overview. *Energy Environ. Sci.* **4:**3913-3929 (2011).

6 Zhao, H, Xia, S, Ma, P, Use of ionic liquids as 'green' solvents for extractions. *J Chem Technol Biotechnol* **80**:1089–1096 (2005)

7 Mäki-Arvela P, Anugwom I, Virtanen P, Sjöholm R and Mikkola JP, Dissolution of lignocellulosic materials and its constituents using ionic liquids-A review. *Ind Crop Prod* **32**: 175-201 (2010).

8 Yoon, LW, Ngoh GC, Chua ASM and Hashim MA, Comparison of ionic liquid, acid and alkali pretreatments for sugarcane bagasse enzymatic saccharification. *J ChemTechnol Biotechnol* **86**:1342–1348 (2011).

9 Diedericks, D, Rensburg E, García-Prado MP and Görgens JF, Enhancing the enzymatic digestibiity of sugarcane bagasse through the application of an ionic liquid in combination with an acid catalyst. *Biotechnol Prog* **28**: 76-84 (2012).

10 Li Q, He YC, Xian M, Jun G, Xu X, Yang JM, Li LZ, Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment. *Bioresour Technol* **100**:3570-3575 (2009).

11 Lee SH, Doherty TV, Linhardt RJ, Dordick JS, Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. *Biotechnol Bioeng* **102**:1368-1376 (2009).

12 Swatloski RP, Scott K, Spear J, Holbrey D and Rogers RD, Dissolution of cellulose with ionic liquids. *J Am Chem Soc* **124**: 4974-4975 (2002).

13 Sant' Ana da Silva A, Lee SH, Endo, T, Bon E, Major improvement in the rate and yield of enzymatic saccharification of sugarcane bagasse via pretreatment with the ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim] [Ac]). *Biores Technol* **102**: 10505-10509 (2011).

14 Zhao H, Baker GA, Cowins JV, Fast enzymatic saccharification of switchgrass after pretreatment with ionic liquids. *Biotechnol Prog* **26**:127-133 (2010).

15 Zavrel M, Bross D, Funke M, Büchs J, Spiess AC, High-throughput screening for ionic liquids dissolving (ligno-)cellulose. *Bioresour Technol* **100**:2580-2587 (2009).

16 Yang B, Wyman CE, Pretreatment: The key to unlocking low-cost cellulosic ethanol. *Biofuel Bioprod Bioref* **2**: 26-40 (2008).

17 Sluiter JB, Ruiz RO, Scarlata CJ, Sluiter AD and Templeton DW, Compositional analysys of lignocellulosic feedstocks. 1. review and description of methods. *J Agric Food Chem* **58**: 9043-9053 (2010).

18 Alvira P, Negro MJ, Sáez F, Ballesteros M, Application of a microassay method to study enzymatic hydrolysis of pretreated wheat straw. *J Chem technol Biotechnol* **85**: 1291-1297 (2010).

19 Romero A, Santos A, Tojo J, Rodríquez A, Toxicity and biodegradability of imidazolium ionic liquids. *J Hazard Mater* **151**: 268-273 (2008).

20 Samaya, IP and Schall, CA, Saccharification of ionic liquid pretreated biomass with commercial enzymes mixtures. *Bioresour Technol* **101**: 3561-3566 (2010).

21 Aroca R, Manisseri C, Li C, Ong M, Schelle HV, Vogel K, Simmons BA and Singh S, Monitoring and analyzing process streams towards understanding ioni liquid pretreatment of switchgrass (*Panicum virgatum* L.). *Bioenerg. Res* **3**:134-145 (2010).

22 Morales de la Rosa S. Campos-Martín JM, Fierro JLG, High-glucose yields from the hydrolysis of cellulose dissolved in ionic liquids. *Chem Eng J* **181-182**: 538-541 (2012).

23 Uju Shoda Y, Nakamoto A, Goto M, Tokuhara W, Noritake Y, Katahira S, Ishida N, Nakashima K, Ogino C, Kamiya N, Short time ionic liquids pretreatment on lignocellulosic biomass to enhance enzymatic saccharification. *Biores Technol* **103**: 446-452 (2012).

24 Klinke HB, Thomsem AB and Ahring BK, Inhibition of ethanol-producing yeast and bacteria by degradation products during pre-treatment of biomass. *Appl Microbiol Biotechnol* **66**: 10-26 (2004).

25 Xiros C, Vafiadi C, Topakas E, Christakopoulos P, Decrement of cellulose recalcitrance by treatment with ionic liquid (1-ethyl-3-methylimidazolium acetate) as a

strategy to enhance enzymatic hydrolysis. *J Chem Technol Biotechnol* **87**: 629-634 (2012)

26 Sun RC, sun XF, Zhang SH. (2001), Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil palm frond fiber, and fast-growing poplar wood. *J Agric Food Chem* **49**:122-5129 (2001).

27 García-Aparicio MP, Ballesteros I, González A, Oliva JM and Negro MJ, Enzymatic hydrolysis of steam–exploded barley straw, in *14th European Biomass Conference, Biomass for Energy, Industry and Climate Protection.* ETA-Renewable Energies, París pp 1182-1185 (2006).

ACKNOWLEDGEMENTS

This work has been partially funded by the Regional Government of Madrid under the R&D Technology Programme/2009 (Project Ref. nº P2009/ENE-1743).

Table 1. Regenerated biomass yield and composition of solids resulting from IL pretreatment at test different conditions.

Temperature (° C)	Time (min)	Regenerated biomass yield (%)	Chemical composition (%)				
			Glucan	Xylan	Galactan	Arabinan	Acid Insoluble Lignin (AIL)
90	30	78.0	37.7 ± 1.7	24.1 ±0.5	1.0 ±0.02	3.4 ±0.2	17.6 ±0.5
	60	79.0	36.6 ±0.1	23.1 ±0.1	1.0 ±0.05	3.7 ±0.04	16.7 ±0.5
110	30	80.3	40.2 ±0.5	24.1 ±0.2	0.6 ±0.03	2.3 ±0.01	16.7 ±0.4
	60	74.8	35.0 ±0.3	21.2 ±0.8	0.6 ±0.03	2.7 ±0.1	18.4 ±0.1
130	30	74.7	45.6 ±1.2	24.7 ±0.3	0.4 ±0.02	2.6 ±0.1	18.6 ±0.1
	60	80.3	34.3 ±0.9	21.1 ±0.7	0.6 ±0.06	2.8 ±0.6	16.3 ±0.5

Table 2. Sugars and phenols analysed in the liquid obtained after IL treatment and precipitation with water (anti-solvent). Data are expressed as mg/100 g raw material

Sugars	PRETREATMENT CONDITIONS					
	90 ºC		110 ºC		130 ºC	
	30 min	60 min	30 min	60 min	30 min	60 min
Glucose	780	550	400	600	210	690
Xylose	nd	nd	420	520	740	nd
4-hydroxybenzoic acid	3.9	6.6	7.8	0.0	7.8	11.7
Vanillin	9.8	19.5	30.8	29.3	35.1	73.3
Syringaldehyde	1.0	1.6	3.3	3.2	8.6	5.5
p-coumaric acid	39.0	46.8	50.3	48.8	23.0	25.7
Ferulic acid	nd	nd	nd	nd	nd	nd

nd. Not detected

Table 3. Overall Sugars yield (g sugar/100 g) from IL-pretreatment of barley straw at varying pretreatment conditions.

Temperature (ºC)	90		110		130	
Time (min)	30	60	30	60	30	60
Overall glucose yield (g/100g raw material)	18.2	21.6	34.8	29.9	35.1	29.6
Overall xylose yield (g/100g raw material)	7.3	10.1	18.7	16.5	15.2	14.2

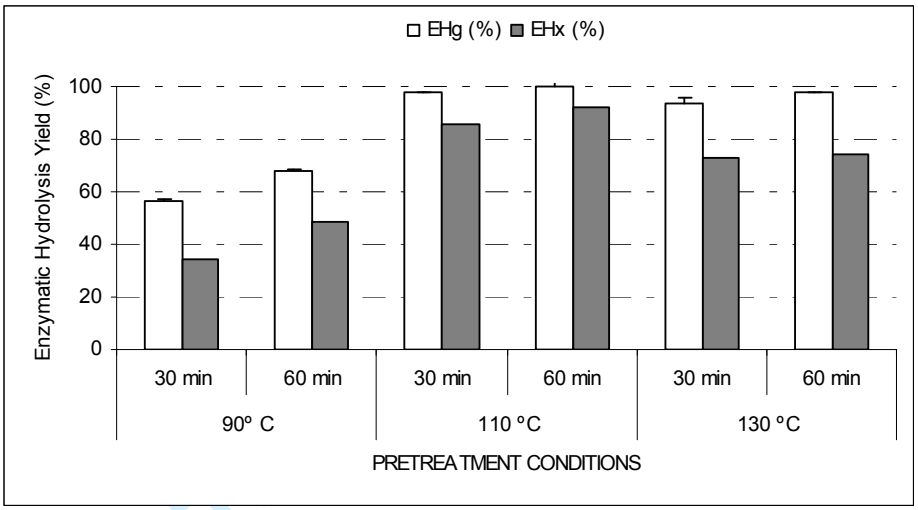

Figure 1. Enzymatic hydrolysis yield (%) of barley straw pretreated by IL at different temperature and residence time conditions (%). Enzymatic hydrolysis conditions: 72 h, 5 % substrate loading. Enzymatic hydrolysis yields (EHg and EHx) were calculated as the ratio of glucose and /or xylose release divided by potential glucose and /or xylose content in solid fraction.