Single-batch, homogeneous phase depolymerization of cellulose catalyzed by a monocomponent endocellulase in ionic liquid [BMIM][Cl]

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1. Introduction

Lignocellulosic biomass is an abundant, renewable, low cost non-food feedstock for biofuel production and a source of C atoms for chemistry alternative to fossil resources [1–4]. The major factor hampering massive exploitation of cellulose (which is the main component of plants biomass), is its well known low reactivity and recalcitrance to chemical processing, which is due to the high crystallinity of cellulose fibrilles. Traditional chemical processes for cellulose modification or derivatization are based on dissolution of cellulose under harsh and environmentally unfriendly conditions, such as hot sodium hydroxyde, or those for the production of viscose, still largely applied worldwide.

A possible greener alternative for the activation of cellulose fibrilles towards an easier hydrolysis and/or chemical derivatization could imply the use of ionic liquids (ILs) [5–7]. Ionic liquids were initially proposed as neoteric solvents for sustainable chemistry due to their unique properties, such as negligible vapour pressure (thus eliminating VOC release in the environment), non-flammability, high dissolving power, the possibility of modulating physico-chemical properties (e.g., hydrophilicity/hydrophobicity) and, in some cases, relatively low toxicity [8–12]. Although these factors do not represent *per se* sufficient conditions for intrinsically green processes [13], they are considered advantageous starting points for the design of environmentally friendly chemical transformations. Along with their benign characteristics as solvents, ILs provide the chemist with new and promising media for synthesis and catalysis on both a laboratory and industrial scale [14–18].

The pioneering work of Rogers and coworkers [19] demonstrated the ability of 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) to dissolve up to 25 wt% cellulose. These authors showed that the IL acts as a pure non-derivatizing solvent able to disrupt the tightly entangled structure of cellulose by replacing

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the intermolecular hydrogen bonds, holding together the polysaccharide chains, with IL/polysaccharide interactions. NMR studies confirmed the key role played by the interactions of carbohydrates OH groups with Cl^- ions of the IL in the dissolution process [20–22]. These findings opened the way to the pretreatment of cellulose with [BMIM][Cl] or other ILs, followed by the conversion of regenerated cellulose into fermentable sugars, possibly via enzymatic reactions, or into new products by chemical derivatization. Indeed, the dissolution of cellulose in ILs and the precipitation of "regenerated cellulose" in aqueous media causes the conversion of type I crystalline cellulose into type II amorphous cellulose [23,24]. From the biocatalytic reaction point of view, the practical consequence of the pretreatment is the generation of a substrate that appears to have a greater accessibility to cellulases for subsequent enzymatic depolymerization. In the last few years, many contributions have highlighted the dramatic enhancement of hydrolysis rate of pretreated cellulose [25–30] by cellulase, thus suggesting that a three-step process – cellulose dissolution, regeneration and cellulase depolymerization – might be eligible for scale-up and potential industrial applications.

An obvious improvement of this strategy, in terms of time saving, energy optimization and sustainability, would be the design of a single-batch process in IL, where the dissolution of cellulose and the enzymatic degradation of the polysaccharidic chains take place in the same medium and reactor. Such an approach would avoid the pretreatment—regeneration steps and the need to remove IL traces before performing the enzymatic hydrolysis in an aqueous buffer. From the process point of view, the one-batch approach would reduce the number of unitary operations, thus leading to saving of time and energy in comparison to multi-batch processes. In principle, the reaction of controlled depolymerization and eventual further chemical modification could be carried out in the same reactor, with considerable advantages in an industrial setting. However, previous studies on biocatalytic degradation of cellulose in [BMIM][Cl] by cellulases from *Trichoderma reesei* showed discouraging results [31]: this work reported a complete loss of enzyme activity due to protein denaturation. Further investigations confirmed the unfriendly character of [BMIM][Cl] towards "traditional" cellulases, as loss of enzymatic activity was reported [32,33], although a significant retention of enzyme activity was observed for methylimidazolium chloride [MIM][Cl] and other ILs with different anions. It is only very recently that a commercial cellulase (Celluclast®) supported onto a polymeric support (Amberlite XAD4) and "protected" by hydrophobic ILs coatings (e.g., 1-butyl-3-methylimidazolium-bis(trifluoromethylsulfonyl)amide, [BMIM][NTf_2]), showed efficient catalytic activity under heterogeneous conditions in the saccharification of cellulose dissolved in [BMIM][Cl] [34].

Comparative evaluation of the cradle-to-gate Life Cycle Assessment (LCA) of [BMIM][Cl] versus the established environmentally friendly *N*-methyl-morpholine-*N*-oxide (NMMO)/H_2O in the industrial dissolution of cellulose for the production of lyocell (which is a viscose-like, man-made fiber), revealed that the IL has the potential for an environmental impact similar to the NMMO/H_2O system, thus renewing the interest in [BMIM][Cl] as a valuable and sustainable medium for industrial cellulose processing [35].

In the present work, we describe a single-batch, homogeneous phase enzymatic depolymerization of cellulose catalyzed by a commercial cellulase (EC 3.2.1.4) in the presence of varying volume ratios of [BMIM][Cl] to buffer. There are several commercial preparations of cellulases available, which can be multi- or mono-component, native or recombinant, from *T. reesei* or *Humicola insolens* or other microorganisms: most of them have been developed for textile and/or pulp and paper applications [36]. We used EGIII (Cel12A), a recombinant mono-component endocellulase from *T. reesei* (see Section 2.1 for details), that showed good stability and activity towards carboxymethyl cellulose (CMC) depolymerization in hydroalcoholic media [36,37]. The IL was used both for cellulose dissolution and cellulase-catalyzed depolymerization, without any pretreatment of cellulose. Our results indicate that a higher specific activity and residual activity after 24 h of incubation is observed in IL than in buffer and thus that a single-batch enzyme-catalyzed depolymerization of cellulose in pure, buffer-free [BMIM][Cl] is actually feasible.

2. Experimental

2.1. Materials

Microcrystalline cellulose from cotton linters was obtained from Lamberti S.p.A. (Albizzate, Italy). Carboxymethylcellulose sodium salt (CMC) was purchased from Fluka (Fluka 21900). Bicinchoninic acid disodium salt hydrate (BCA) was purchased from Sigma-Aldrich (D8284).

The ionic liquid [BMIM][Cl] was purchased from Fluka and used without further purification. The water content in the ionic liquid was less than 1% and this should account for the enzymatic hydrolysis of cellulose that occurs in this medium (*see below*). The industrial enzyme preparation of mono-component endoglucanase EGIII (Cel12A), in a concentrated granular form, from *T. reesei* used in this study was IndiAge® Super GX Plus, referred as Super GX Plus, from Genencor Intl (now a subsidiary of Dupont). The main characteristics of this enzyme are: 930 U/g, 248 mg protein/g commercial cellulase product. The enzymatic activity was measured by the CMC method previously reported [36]. The enzyme shows an optimum activity at acidic pH, thus 50 mM sodium acetate buffer pH 4.8 was used in all experiments.

All other reagents were of analytical grade and used without further purification.

2.2. Determination of reducing-ends by the BCA method

Quantification of the enzymatic depolymerization of cellulose was based on the estimation of reducing sugars produced during hydrolysis by the BCA method ([38] and references therein). Two stock solutions were prepared and stored separately for one month at 4 °C. Solution A: 0.971 g of disodium 2,2-bicinchoninate, 27.14 g of Na_2CO_3 and 12.1 g of $NaHCO_3$ were dissolved in 500 mL of distilled water. Solution B: 0.624 g of $CuSO_4 \cdot 5H_2O$ and 0.631 g of L-serine were dissolved in 500 mL of distilled water. BCA working solution was freshly prepared before use by mixing equal volumes of solution A and solution B.

In details: 1 mL of BCA working solution was added to 1 mL of sample in a vial and incubated at 70 °C for 30 min. The vials were cooled at room temperature, 1 mL of mixture was transferred to a cuvette and the absorbance was determined at 560 nm.

A calibration curve was established by using a glucose solution as standard in the 0–82 μM concentration range. In all cases, the contribution of the added protein to color development was measured and subtracted from the final value.

2.3. Measurements of cellulase activity

The enzyme activity was determined on a standard solution of 1% (w/v) CMC in distilled water [36]. 500 μL of CMC solution were incubated in a 2 mL vial at 40 °C for 10 min. Then, 500 μL of cellulase, previously diluted in buffer, were added; the mixture was vortexed and the vial incubated for 10 min at 40 °C. The reaction was stopped by adding 1 mL of the BCA working solution (the inhibition of the enzymatic reaction is due to the high alkaline pH) and the determination of the reducing ends starts simultaneously. The vial was vortexed again and incubated at 70 °C for 30 min. After

cooling at room temperature, 1 mL of mixture was transferred to a 3 mL cuvette. The absorbance at 560 nm was determined *versus* a blank composed of 500 μL of buffer, 500 μL of 1% CMC solution or 500 μL of cellulase and 1 mL of BCA working solution. Product concentration was determined based on a calibration curve established with glucose as a standard.

One enzymatic unit (U) is defined as the amount of enzyme which liberates 1 μmol of glucose equivalent from CMC in one min at 40 °C.

2.4. Measurements of cellulase stability in [BMIM][Cl] versus temperature and time of incubation

For the evaluation of temperature stability of the enzyme preparation, 5 mg of cellulase Super GX Plus was incubated in 250 μL of [BMIM][Cl] (or buffer for the control reaction) for 1 h under magnetic stirring at different temperatures ranging from 75 to 90 °C. A 10 μL aliquot was withdrawn and diluted to 1 mL with buffer: 500 μL of this solution were immediately used to assay the residual enzymatic activity (see above).

The time stability of cellulase at a fixed temperature of 75 °C was assessed as follows: 5 mg of cellulase were incubated in 250 μL of [BMIM][Cl] (or buffer for control reaction) under magnetic stirring at 75 °C for up to 24 h. An aliquot (10 μL) was withdrawn and diluted to 1 mL with buffer; 500 μL of this solution were used for cellulase activity determination on CMC (see above).

2.5. Enzymatic hydrolysis of cellulose

In a 1.5 mL screw-capped vial, 2.5 mg of cellulose were suspended in 1 mL of buffer/IL and allowed to equilibrate at 75 °C. In different experiments, the volume ratio of IL to buffer was varied but cellulose concentration (2.5 mg/mL) was kept constant. The reaction was started by adding 2.5 mg equivalent to 2.33 U (or 25 mg equivalent to 23.3 U) of cellulase preparation and the mixture was vigorously mixed by magnetic stirring. Samples (10 μL) were periodically withdrawn and reducing-ends determined by the BCA method. Controls were prepared similarly but without cellulose. Assays were performed at least three times each and mean values were calculated.

2.6. Fluorescence measurements

Protein fluorescence measurements were performed in a Jasco FP-750 instrument using a thermostated cell holder at 25 °C. In order to measure fluorescence at this standard temperature on a liquid sample, 1% water was added to the pure ionic liquid. In details: 10 mg of enzyme sample were fully dissolved by stirring for 2 h at 75 °C in 1 mL of solvent (IL containing 1% of water or buffer), 20 μL of this solution were diluted with the same solvent to a final volume of 800 μL (0.25 mg protein/mL). Protein emission spectra were recorded from 300 to 400 nm with excitation at 280 nm (excitation slit of 5 nm, emission slit of 10 nm) [39]. The emission of a blank control without enzyme was subtracted from all measurements in order to correct for the contribution of the imidazolium ring to the overall enzyme fluorescence spectra.

3. Results and discussion

3.1. Effect of temperature

The effect of temperature on cellulase activity of Super GX Plus in [BMIM][Cl] was investigated and compared to the value obtained in buffer as a control. Super GX Plus, being in granular form, was assayed after 2 h at 75 °C in IL because of its slower dissolution at that temperature after 60 min incubation.

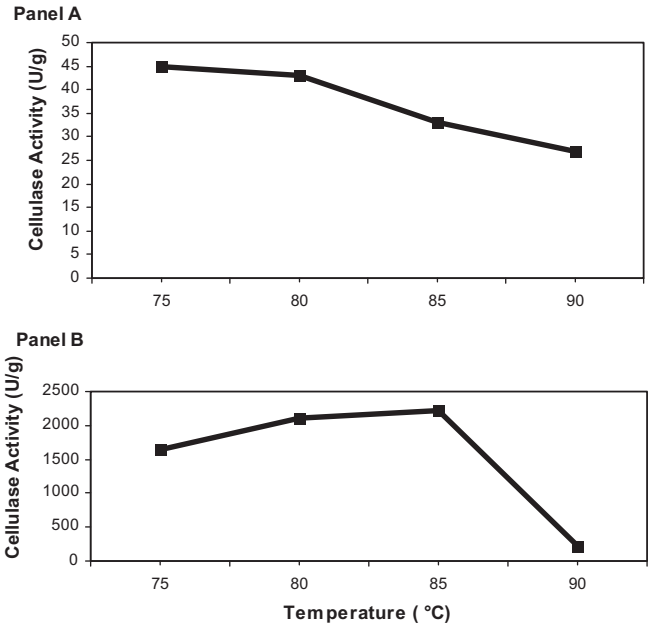

Fig. 1. Effect of temperature on cellulase residual activity in buffer (Panel A) and in [BMIM][Cl] (Panel B). The cellulase activities were measured after incubation of 5 mg of enzyme preparation in 250 μL of [BMIM][Cl] (or 50 mM sodium acetate buffer pH 4.8 for control reaction) for 1 h at different temperatures. A 10 μL aliquot of this solution was withdrawn, diluted with suitable buffer and used for cellulase activity assay on CMC as indicated in Section 2.3. For Super GX Plus in [BMIM][Cl] at 75 °C the reported data have been recorded after 2 h of incubation because the commercial preparation takes this long time to be fully dissolved in IL (see text). Error bars are smaller than the symbols used (standard deviation is <5%).

As can be observed from Fig. 1A, the specific activity in buffer of the cellulase used in this work shows a significant decrease after 1 h incubation at temperatures ≥ 75 °C. Conversely, the activity of the enzyme in [BMIM][Cl] increased up to 85 °C reaching a value 2–3 times higher than the corresponding activity in buffer at 40 °C (Fig. 1B). Thus, the ionic liquid appears to have the dual effect of increasing the enzymatic activity and protecting the enzyme against thermal denaturation. As a consequence, higher reaction temperatures than those used in an aqueous medium can be used for the biocatalytic depolymerization of cellulose in IL. It is interesting to notice that at 90 °C Super GX Plus in IL exhibits a marked decrease of stability (Fig. 1B), thus suggesting the 75–85 °C temperature range as optimum for dissolution-hydrolysis of cellulose. The experimental values determined at 75 °C deserve a specific comment. At that temperature, the enzyme in a patented granular form (commercially referred to as Enzoguard®) dissolves more slowly in IL than in buffer, thus it expresses less activity after the same time span. Super GX Plus needs about 2 h for full dissolution in [BMIM][Cl] at 75 °C, while it dissolves instantly in buffer. Complete dissolution of the granules was established by visual assessment and levelling off of cellulase activity. Also, the observed effect of [BMIM][Cl] on Super GX Plus cellulase activity at high temperature (Fig. 1) is probably related to its ability to fully dissolve the enzyme preparation.

Protein fluorescence spectra of the cellulase after 1 or 2 h of incubation in buffer *versus* [BMIM][Cl] was compared (the reported fluorescence spectra have been corrected for contributions due to buffer or ionic liquid): a dramatic quenching of fluorescence signal was revealed in IL, as shown in Fig. 2.

This change is due to the significant alteration of the microenvironment surrounding the tryptophan residues and, therefore, cannot be related to protein unfolding phenomena, since the enzyme is still active in the ionic liquid. This result differs from what was observed by Turner et al. [31]: in that case the change

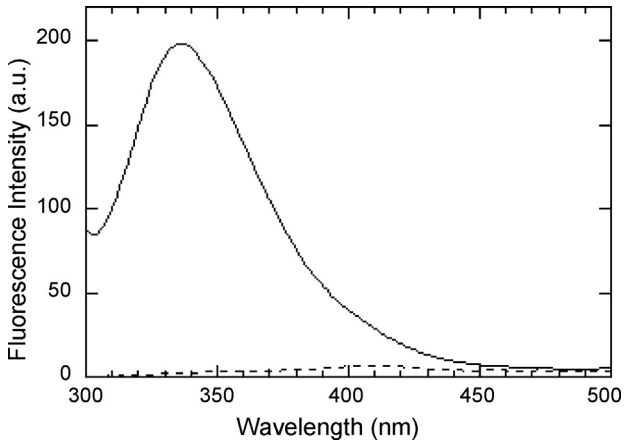

Fig. 2. Fluorescence spectra of Super GX Plus in buffer (50 mM sodium acetate pH 4.8, continuous line) and in [BMIM][Cl] (dotted line), following subtraction of the pure solvent contribution. Conditions: 0.25 mg protein/mL, 25 °C. The IL contains 1% of water to allow solubilization at the assay temperature.

in protein fluorescence was directly proportional to the enzymatic activity loss. This discrepancy is probably due to the different origin and preparation of the *T. reesei* cellulases used by the two laboratories, i.e. a native enzyme in a lyophilized-KH_2PO_4 complexed physical form from Sigma vs. a recombinant protein in a concentrated granules form from Danisco-Genencor.

The results obtained in the present work in [BMIM][Cl] and those reported in the literature [31–33] indicate that the design of biocatalytic processes in ILs should carefully consider the type of cellulase used, as a considerable variability can be observed in enzyme stability/activity, presumably related to the microbial source of origin and/or to the introduction of genetic engineering manipulations.

3.2. Enzyme stability at 75 °C in [BMIM][Cl]

The results of the previous section prompted us to investigate the stability time-course of the enzyme in pure [BMIM][Cl] at 75 °C. This temperature was chosen because: (i) it is the lowest at which [BMIM][Cl] is in liquid state; (ii) it efficiently solubilizes cellulose; (iii) it seems to maintain the enzyme active over extended periods of time. Results are shown in Fig. 3.

The enzyme preparation showed a time-dependent inactivation when incubated in buffer while no change in activity was apparent up to 6 h in ionic liquid (Fig. 3). A significant decrease in activity after 24 h in IL was observed (10% of the activity measured at 2 h, not shown). In any case, it should be pointed out that the enzyme preparation showed higher residual activity at 75 °C in [BMIM][Cl] compared to buffer even after 24 h of incubation (~350 U/g in ionic liquid vs. 8–12 U/g in buffer solution), further demonstrating that this highly viscous IL protects/preserves the protein activity against inactivation more effectively than an aqueous medium. The stabilization of the protein in [BMIM][Cl] at high temperature represents the prerequisite for the one-pot solubilization and enzymatic cellulose hydrolysis process.

3.3. Effect of solvent on enzymatic cellulose hydrolysis

The effect of solvent composition (IL/water ratio) on the outcome of the enzymatic depolymerization of cellulose was tested. Firstly, blank experiments without enzyme were carried out and demonstrated that IL or mixture of IL/buffer did not cause cellulose hydrolysis in the absence of enzyme. To perform enzymatic hydrolysis, cellulose was first dissolved in IL/buffer at 75 °C, followed by cellulase addition. After 24 h, the reaction mixture was evaluated using the BCA assay for reducing-ends determination.

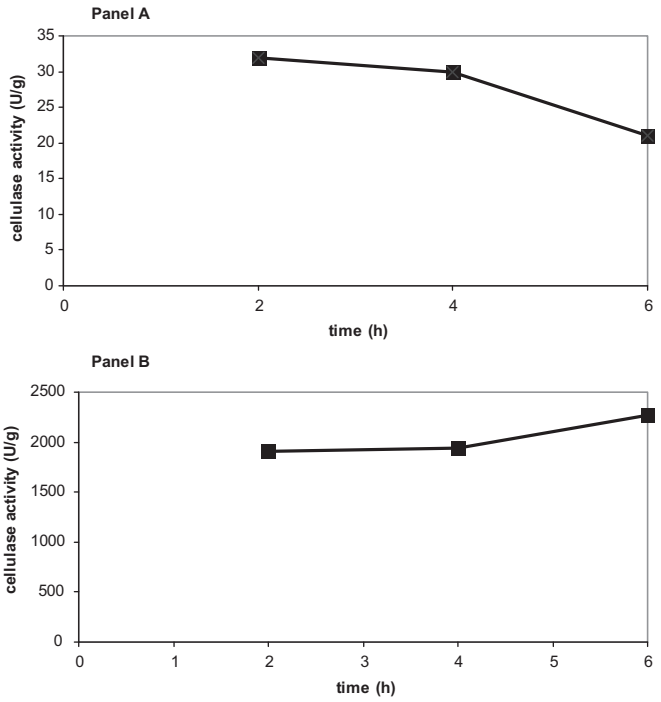

Fig. 3. Time course of cellulase stability at 75 °C in 50 mM sodium acetate pH 4.8 buffer (Panel A) and in [BMIM][Cl] (Panel B) for Super GX Plus (■). The reactions were performed as indicated in Section 2. The standard deviation (<5%) is such a small value that the error bars are smaller than the symbols used.

The data, reported after substraction of blank experiments without cellulose, are shown in Fig. 4, where the conversion of cellulose is expressed as reducing ends glucose equivalent concentration (μM) per enzyme unit as a function of solvent composition. Setting 100% conversion for Super GX Plus in buffer the enzyme is surprisingly active in pure IL showing 41.5% of its activity in buffer. To the best of our knowledge, such experimental evidence of high enzymatic hydrolysis in pure [BMIM][Cl] has not been previously reported. Intringuisly, the enzyme does not show any enzymatic activity at IL/buffer 50/50 ratio.

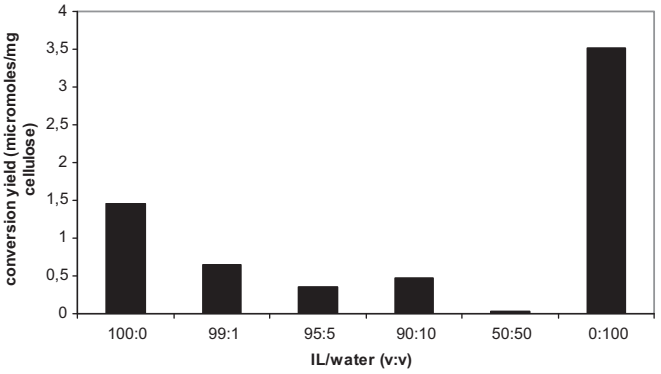

Fig. 4. Effect of the concentration of ionic liquid -expressed as volume ratios of IL/buffer (50 mM sodium acetate pH 4.8) for Super GX Plus on the conversion of cellulose at 1/10 substrate/commercial enzyme preparation (w/w) after 24 h reaction. The conversion yield after 24 h reaction at 75 °C is expressed as micromoles reducing end glucose equivalent/mg of starting cellulose (100% of conversion is the equivalent of 0.667 μmol of reducing end glucose equivalent/enzyme unit in buffer). The reactions were performed at 75 °C using 2.5 mg of cellulose, 25 mg of enzyme preparation and 1 mL of solvent as indicated in Section 2.5. The standard deviation was <5%.

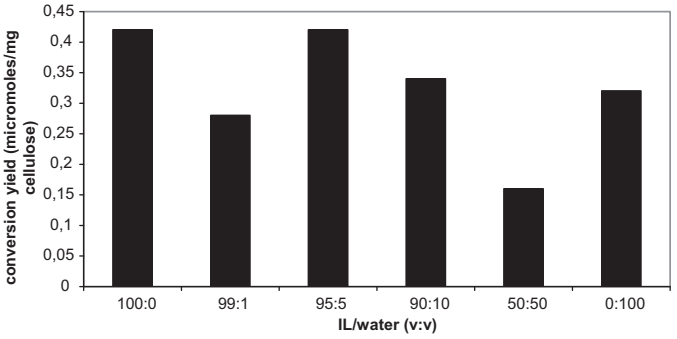

Fig. 5. Effect of a lower cellulose/enzyme ratio (w/w of 1/1) on cellulose saccharification versus solvent composition expressed as volume ratios of IL/buffer (50 mM sodium acetate pH 4.8) for Super GX Plus. The conversion yield after 24 h reaction at 75 °C is expressed as micromoles reducing end glucose equivalent/mg of starting cellulose. The reactions were performed using 2.5 mg of cellulose, 2.5 mg of cellulase (ratio (w/w) 1:1 substrate/enzyme preparation) and 1 mL of solvent (IL/water) as indicated in Section 2. The standard deviation was <5%.

3.4. Effect of cellulose/enzyme ratio (w/w) on enzymatic hydrolysis

The work described in the previous sections was carried out by using a 1/10 (w/w) cellulose/commercial enzyme preparation (S/E) ratio. In order to elucidate the influence of the amount of enzyme on the reaction kinetics, the reducing sugars released during enzymatic hydrolysis of cellulose at a different S/E ratio, namely 1:1 (w/w), were also evaluated. Along with the predictable increase of conversion in the case of S/E = 1/10 (Fig. 4) *versus* 1/1 (Fig. 5), the data of Fig. 5 clearly indicate that the enzyme from *T. reesei* is very sensitive to the S/E ratio. At the S/E = 1:1 ratio (Fig. 5), the conversion yield is not significantly affected by the reaction medium, while when an excess of cellulase is used (Fig. 4), the highest conversion yields are observed in pure solutions of IL or buffer. Indeed, the time course of cellulose hydrolysis in [BMIM][Cl] *versus* buffer (not shown) indicates that the cellulase conserves enzymatic activity during the entire course of the reaction (24 h) at 75 °C.

4. Conclusions

This work demonstrates that [BMIM][Cl] might represent a suitable medium for the enzymatic depolymerization of cellulose in a one-batch process. It suggests that a preliminary careful choice of cellulase preparation and reaction conditions is required. Indeed, the recombinant, monocomponent endocellulase EGIII (Cel12A) from *T. reesei* tested in this work (commercially known as IndiAge Super GX Plus) was not denatured by the ionic liquid. Interestingly, this enzyme showed exceedingly good performance for the depolymerization of dissolved cellulose in [BMIM][Cl] without the usual pretreatment, known as dissolution-regeneration of cellulose in its amorphous form. Super GX Plus is fully dissolved in [BMIM][Cl] and retains a great portion of its activity in this ionic liquid for ≥6 h. This same mono-component endocellulase by recombinant DNA manipulation from the natural cellulase complex of *T. reesei* was previously shown to be the most stable and active in alcoholic reaction medium containing high percentage of 2-propanol or ethanol [36].

In conclusion, the approach outlined in the present work could be amenable of scaling-up and innovative industrial applications for the efficient one-batch conversion of inexpensive cellulosic materials into derivatives (biofuels, derivatized cellulose, monosaccharides for fine chemicals, etc.) with high potential commercial interest and in the framework of environmentally "green" chemistry. The more friendly ILs based on 1-ethyl-3-methylimidazolium acetate diethylphosphate were also studied and the manuscript is currently in preparation.

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References

[1] J.B. Binder, R.T. Raines, J. Am. Chem. Soc. 131 (2009) 1979.
[2] J.B. Binder, R.T. Raines, Proc. Natl. Acad. Sci. 107 (2010) 4516.
[3] P. Wang, H. Yu, S. Zhan, S. Wang, Bioresour. Technol. 102 (2011) 4179.
[4] Z. Zhang, Q. Wang, H. Xie, W. Liu, Z. Zhao, Chem. Sus. Chem. 4 (2011) 131.
[5] S. Zhu, Y. Wu, Q. Chen, Z. Yu, C. Wang, S. Jin, Y. Ding, G. Wu, Green Chem. 8 (2006) 325.
[6] I. Kilpeläinen, H. Xie, A. King, M. Granstrom, S. Heikkinen, D.S. Argyropoulos, J. Agric. Food Chem. 55 (2007) 9142.
[7] A. Pinkert, K.N. Marsh, S. Pang, M.P. Staiger, Chem. Rev. 109 (2009) 6712.
[8] S. Stolte, J. Arning, U. Bottin-Weber, M. Matzke, F. Stock, K. Thiele, M. Uerdingen, U. Welz-Biermann, B. Jastorff, J. Ranke, Green Chem. 8 (2006) 621.
[9] R. Anand Kumar, N. Papaïconomou, J.M. Lee, J. Salminen, D.S. Clark, J.M. Prausnitz, Environ. Toxicol. 24 (2009) 388.
[10] M. Matzke, S. Stolte, K. Thiele, T. Juffernholz, J. Arning, J. Ranke, U. Welz-Biermann, B. Jastorff, Green Chem. 9 (2007) 1198.
[11] C. Pretti, C. Chiappe, D. Pieraccini, M. Gregori, F. Abramo, G. Monni, L. Intorre, Green Chem. 8 (2005) 238.
[12] D. Zhao, Y. Liao, Z. Zhang, Clean 35 (2007) 42.
[13] J. Ranke, S. Stolte, R. Störmann, J. Arning, B. Jastorff, Chem. Rev. 107 (2007) 2183.
[14] J.S. Wilkes, Ionic Liquids Industrial Applications to Green Chemistry, in: R.D. Rogers, K.R. Seddon (Eds.), ACS Symp. Ser., vol. 818, American Chemical Society, Washington, DC, 2002, pp. 214–229.
[15] N.V. Plechkova, K.R. Seddon, Chem. Soc. Rev. 37 (2008) 123.
[16] J.P. Hallett, T. Welton, Chem. Rev. 111 (2011) 3508.
[17] P. Wasserscheid, in: P.T. Anastas (Ed.), Handbook of Green Chemistry, vol. 6, Wiley-VCH, Weinheim, 2010, pp. 65–91.
[18] P. Lozano, T. De Diego, M. Vaultier, L.L. Iborra, Ionic Liquid Applications: Pharmaceuticals, Therapeutics and Biotechnology, in: S. Malhotra (Ed.), ACS Symp. Ser., vol. 1038, American Chemical Society, Washington, DC, 2002, pp. 181–196.
[19] R.P. Swatloski, S.K. Spear, J.D. Holbrey, R.D. Rogers, J. Am. Chem. Soc. 124 (2002) 4974.
[20] J.S. Moulthrop, R.P. Swatloski, G. Moyna, R.D. Rogers, Chem. Commun. (2005) 1557.
[21] R.C. Remsing, R.P. Swatloski, R.D. Rogers, G. Moyna, Chem. Commun. (2006) 1271.
[22] L. Feng, Z.L. Chen, J. Mol. Liq. 142 (2008) 1.
[23] M. Deng, Q. Zhou, A. Du, J. van Kasteren, Y. Wang, Mater. Lett. 63 (2009) 1851.
[24] A.P. Dadi, C.A. Schall, S. Varanasi, Appl. Biochem. Biotechnol. 136–140 (2007) 407.
[25] A.P. Dadi, S. Varanasi, C.A. Schall, Biotechnol. Bioeng. 95 (2006) 904.
[26] C.H. Kuo, C.K. Lee, Carbohydr. Polym. 77 (2009) 41.
[27] H. Zhao, C.L. Jones, G.A. Baker, S. Xia, O. Olubajo, V.N. Person, J. Biotechnol. 139 (2009) 47.
[28] T.A.D. Nguyen, K.R. Kim, S.J. Han, H.Y. Cho, J.W. Kim, S.M. Park, J.C. Park, S.J. Sim, Bioresour. Technol. 101 (2010) 7432.
[29] S.H. Ha, N.L. Mai, G. An, Y.M. Koo, Bioresour. Technol. 102 (2011) 1214.
[30] K. Wang, H.Y. Yang, F. Xu, R.C. Sun, Bioresour. Technol. 102 (2011) 4524.
[31] M.B. Turner, S.K. Spear, J.G. Huddleston, J.D. Holbrey, R.D. Rogers, Green Chem. 5 (2003) 443.
[32] S. Bose, D.W. Armstrong, J.W. Petrich, J. Phys. Chem. B 114 (2010) 8221.
[33] P. Engel, R. Mladenov, H. Wulfhorst, G. Jäger, A.C. Spiess, Green Chem. 12 (2010) 1959.
[34] P. Lozano, B. Bernal, J.M. Bernal, M. Pucheault, M. Vaultier, Green Chem. 13 (2011) 1406.
[35] S. Righi, A. Morfino, P. Galletti, C. Samori, A. Tugnoli, C. Stramigioli, Green Chem. 13 (2011) 367.
[36] S. Cheroni, C. Formantici, Y.M. Galante, Enz. Microb. Technol. 47 (2010) 348.
[37] U. Arunachalam, Jt Kellis Jr., Biochemistry 35 (35) (1996) 11379–11385.
[38] Y.H.P. Zhang, L.R. Lynd, Biomacromolecules 6 (2005) 1510.
[39] L. Pollegioni, S. Iametti, D. Fessas, L. Caldinelli, L. Piubelli, A. Barbiroli, M.S. Pilone, F. Bonomi, Protein Sci. 12 (2003) 1018.