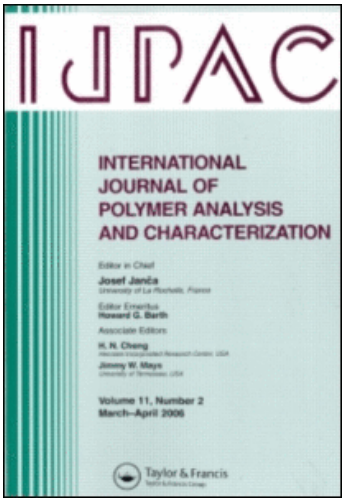

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INFLUENCE OF IONIC LIQUID ON HYDROLYZED CELLULOSE MATERIAL: FT-IR SPECTROSCOPY AND TG-DTG-DSC ANALYSIS

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Three different ionic liquids (IL), 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl), and 1-butyl-3-methylpyridinium chloride ([BMP]Cl), were used as reaction media in hydrolysis reaction of poplar seed floss (as cellulose material) with cellulase. Hydrolysis kinetics of the IL-treated cellulose material was significantly enhanced. The initial hydrolysis rates for IL-treated cellulose material were greater than that of non-treated cellulose material. This behavior seems to be due to* synergetic *effects of ionic liquid and cellulase upon cellulose material. Structural modifications were investigated by Fourier transform-infrared spectroscopy (FT-IR). Thermal properties of poplar seeds floss were also evaluated by TG-DTG-DSC simultaneous analysis.

***Keywords*:** Cellulose material; FT-IR spectroscopy; Hydrolysis; Ionic liquid; TG-DTG-DSC analysis

INTRODUCTION

The limited reserves of fossil fuels and global climate changes have increased attention to the use of renewable biomaterials for energy production. Research has focused on ethanol production from cellulose, an almost inexhaustible polymeric raw biomaterial.[1] For bioproduction of ethanol, cellulose should ultimately be hydrolyzed to glucose for fermentation.

In its natural state, cellulose is highly crystalline in structure with individual cellulose polymer chains held together by strong hydrogen bonding and van der Waals forces. The individual cellulose chains are linear condensation polymer molecules made up of anhydroglucose units joined together by β-1,4-glycosidic bonds[2] with degrees of polymerization ranging from 1000 to 15,000 units. In general, neither the water molecules nor the catalysts for hydrolysis (like cellulase enzymes) are able to easily penetrate the crystalline matrix.[3]

Enzymatic saccharification processes require the dissolution of cellulose in a solvent to facilitate the access of cellulase to cellulosic substrates. Ionic liquids (ILs), a new class of cellulose-dissolving solvents[4] and new reaction media for biocatalysis,[5] are potential solvents for the enzymatic saccharification of cellulose. However, the

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significant decrease in cellulase activity in the presence of cellulose-dissolving ILs[6] requires that a cumbersome recovery process is necessary to retrieve the regenerated cellulose produced by the pretreatment of cellulose with ILs prior to enzymatic saccharification.[7] To simplify the entire process, it is necessary to develop ILs compatible with both cellulose solubility and cellulase activity.

The application of IL as solvents in carbohydrate chemistry has recently been reviewed.[8,9] Some ionic liquids, especially those containing Cl^- anion, dissolve cellulose.[10] Ionic liquids have the ability to dissolve large amounts of cellulose at very mild conditions, and the feasibility of recovering nearly 100% of the used IL to its initial purity makes them attractive.[7,11,12] Recently, cellulose solubilities of up to 39, 25, and 10% (w/w) have been reported for the ILs 3-methyl-N-butylpyridinium chloride,[11] 1-*n*-butyl-3-methylimidazolium chloride ([BMIM]Cl),[4] and 1-allyl-3-methylimidazolium chloride ([AMIM]Cl),[13] respectively.

Formulators of cellulose chemicals and soft fibrous structures are always looking for additional types of fibers in order to improve performance or reduce cost. Soft fibrous structures have conventionally been made with wood pulp fibers. More recently, synthetic fibers have been used. Another source is seed hairs, which may protect a seed and/or aid in the transport of a seed present on a plant. Many plants have seed hairs, and they have a wide range of morphology and chemical properties. For example, the seed hairs may be in the form of fibers, namely, seed hair fibers. Such seed hair fibers may have a high length-to-diameter ratio. Methods of separating and cleaning cotton staple fibers and/or cotton linter fibers, for example, are well known and effective. However, they are unsuitable for use with other types of seeds.

Individualized seed hairs may be converted into chemical derivatives including but not limited to cellulose derivatives. Individualized seed hairs may also be used in their physical form, usually fibrous, herein referred to as seed hair fibers, as a component of fibrous structures. In our study, poplar seed floss without any modification has been enzymatically hydrolyzed in the presence of different ionic liquids: 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl), 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), and 1-butyl-4-methylpyridinium chloride ([BMP]Cl). The combined action of the ionic liquids and cellulase in conditions of hydrolysis reaction (40°C, pH 4.8, 120 h) was studied in order to determine the effect of ionic liquids on hydrolysis rate of poplar seed floss. Structural modifications were investigated by Fourier transform-infrared spectroscopy (FT-IR). Thermal properties of poplar seeds floss were also evaluated by thermogravimetric-derivative thermogravimetric-differential scanning calorimetry (TG-DTG-DSC) simultaneous analysis

EXPERIMENTAL SECTION

Materials

Sodium acetate dihydrate, sodium potassium tartrate (Rochelle salt), 3,5-dinitrosalicylic acid (DNS), sodium hydroxide, phenol, sodium metabisulfite, acetic acid, and ethanol were obtained from Sigma-Aldrich Ltd. (Germany). Ionic liquids [BMIM]Cl, [EMIM]Cl, and [BMP]Cl were purchased from Fluka and used without further purification (see Figure 1). Cellulase from *Aspergillus niger* (BioChemika,

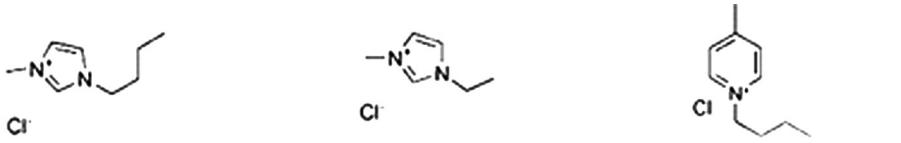

Figure 1. Structure of ionic liquids (ILs).

Fluka) was used in hydrolysis reactions. The poplar seed floss (PSF) was used without any preliminary treatment.

Cellulase-Catalyzed Hydrolysis of PSF

The poplar seed floss samples (coded PSF as presented in Table I) were hydrolyzed without or with ionic liquid in vials at 40°C for 120 h in a WNB 7-45 shaker water bath, using a PSF/IL ratio of 1:15 w/w.

The total vial volume was 5 mL with cellulase concentration of 30 FPU/g cellulose material and dried PSF addition of 2 mg/mL. The mixture was buffered with 50 mM acetate acid-sodium acetate, pH 4.8. All PSF samples were hydrolyzed using the same cellulase stock solution. The reaction was monitored by periodically withdrawing samples and measuring release of soluble-reducing sugars. The control hydrolysis reactions were run concurrently with experiments in the presence of ILs to eliminate potential differences in temperature history or enzyme loading. Yield of reducing sugars from PSF sample hydrolysis was calculated as follows:

$$Yield\ (\%) = \frac{reducing\ sugars\ weight}{substrate\ cellulose\ material\ weight} \times 100$$

Analysis Methods

Poplar seed floss (PSF) is composed of 62.07% cellulose, 17.04% lignin, and 2.5% ash. Chemical analysis of PSF was performed according to the TAPPI methods.

Reducing sugar was measured by the DNS assay using D-glucose as a standard.[14] Triplicate determinations were made for each hydrolyzate sample.

Table I. Codes of poplar seed floss (PSF) samples

Sample code	Ionic liquid (IL)	Poplar seed floss
PSF	—	initial
HPSF	—	hydrolyzed without IL
HPSF-IL1	[BMIM]Cl	hydrolyzed
HPSF-IL2	[EMIM]Cl	hydrolyzed
HPSF-IL3	[BMP]Cl	hydrolyzed

Cellulase activity was determined by the standard filter paper assay and expressed as filter paper units per gram of glucan (FPU).[15] One FPU is defined as the enzyme that releases 1 μmol of glucose equivalents per minute from Whatman No. 1 filter paper.

All PSF samples were analyzed by FT-IR. FT-IR spectra were registered on a Bruker Vertex 70 spectrophotometer. The spectra (4000–400 cm^{-1}) were recorded with a resolution of 4 cm^{-1} and 64 scans per sample. About 2.0 mg samples were prepared by mixing with 120 mg of spectroscopic grade KBr then pressed to produce 13 mm diameter pellets.

The thermal analyses were performed with a Netzsch STA 449 F1 thermal analyzer (Germany). Samples (≈5 mg) were placed in Al_2O_3 crucibles hermetically closed with lids and heated under nitrogen from room temperature up to 600°C at a 10°C/min rate of temperature increase. TG and DSC curves recorded with a ±0.5°C precision were analyzed with Netzsch Proteus analysis software.

RESULTS AND DISCUSSION

Cellulase-Catalyzed Hydrolysis of PSF

It was shown that the structure of cellulose material regenerated from used ionic solutions by addition of water is less crystalline than that of the original untreated cellulose.[16] The influence of ionic liquids on the enzymatic hydrolysis of PSF with cellulase was evaluated. The PSF solubilization in ionic liquid reduced the crystallinity degree with a positive impact on the rates of enzymatic hydrolysis. It was found that the enzymatic hydrolysis rate was improved significantly by addition of ionic liquid (Figures 2 and 3).

It seems that the PSF samples exhibiting lower crystallinity and higher cellulase adsorption were hydrolyzed by cellulase much faster in the presence of ILs than the samples without ILs. After 120 h of enzymatic reaction, the hydrolysis rate of PSF with [BMP]Cl was greater than that obtained in the presence of [EMIM]Cl or [BMIM]Cl.

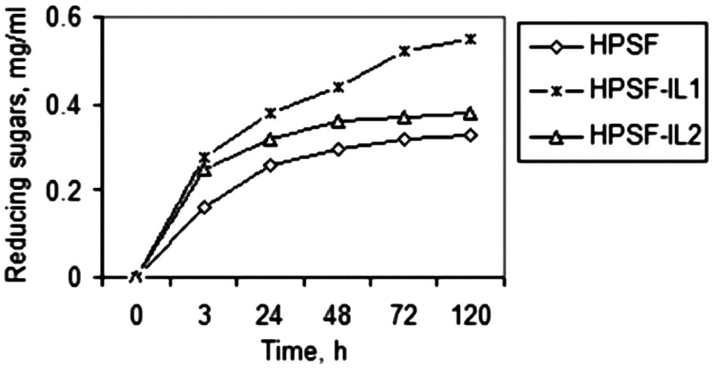

Figure 2. Effect of [BMIM]Cl and [EMIM]Cl on reducing sugar release during PSF saccharification.

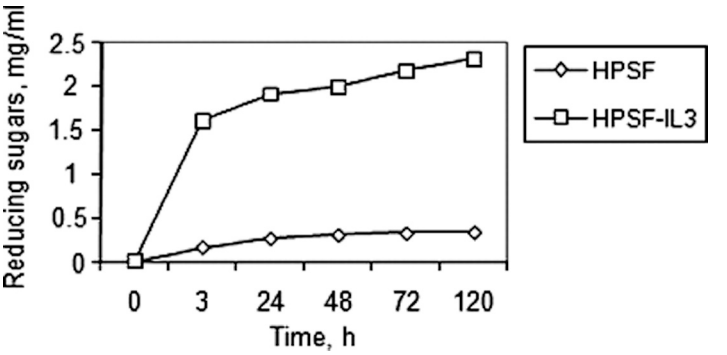

Figure 3. Effect of [BMP]Cl on reducing sugar release during PSF saccharification.

FT-IR Analysis of Hydrolyzed PSF

Figure 4 shows the FT-IR spectra of initial PSF and after enzymatic hydrolysis (HPSF). It appears that peak intensity was lower after hydrolysis, mainly in the presence of IL, indicating degradation of cellulose. The FT-IR spectra show specific cellulose peaks around 1000–1200 cm^{-1}.[17,18] The band near 1160 cm^{-1} is representative of the antisymmetric bridge stretching of C–O–C groups in cellulose and hemicelluloses, while the band near 1318 cm^{-1} can be ascribed to CH_2– wagging vibrations in the cellulose and hemicelluloses. A decrease in intensity of both the 897 cm^{-1} band corresponding to the β-linkages, especially in hemicelluloses, and the band at 1635–1640 cm^{-1}, attributed to the absorbed water bending vibrations, was observed after PSF hydrolysis in the presence of ionic liquid.

As shown in Figure 4, the absorption band at 1430 cm^{-1} was strong for PSF before enzymatic hydrolysis, but weak for degraded substrate.

The changes of cellulose structure in PSF after enzymatic hydrolysis without and with ionic liquid are revealed in the infrared spectra. From these spectra, three infrared ratios were calculated: (1) A_{1430}/A_{897}, which is referred to as the crystallinity index[19] or lateral order index (LOI),[20] (2) A_{1372}/A_{2900}, known as the total crystallinity index (TCI),[21] and (3) A_{3308}/A_{1330}, known as hydrogen-bond intensity (HBI),[20,22] closely related to the crystal system and the degree of intermolecular regularity, as well as the amount of bound water. These parameters are presented in Table II.

A high index value indicates high crystallinity and an ordered structure for substrate. As shown in Table II, after enzymatic hydrolysis, the LOI decreased for PSF from 2.72 to 1.97 without IL to 1.60 with [BMP]Cl. The TCI significantly increased after enzymatic hydrolysis, this evolution being significant in the presence of [BMIM]Cl and [BMP]Cl. It seems that a part of the crystalline structure of cellulose was transformed into amorphous form in the presence of ionic liquid. As a consequence, the fragmental and porous cellulose materials with amorphous structure provided more surfaces for enzymes to attack, which is shown by the enzymatic hydrolysis rate of PSF in the presence of ionic liquid.

LOI and HBI parameters show a significant decrease in comparison with the initial PSF. The lower values of LOI were obtained for PSF after enzymatic hydrolysis in the presence of ILs. In the presence of [EMIM]Cl, TCI had lower

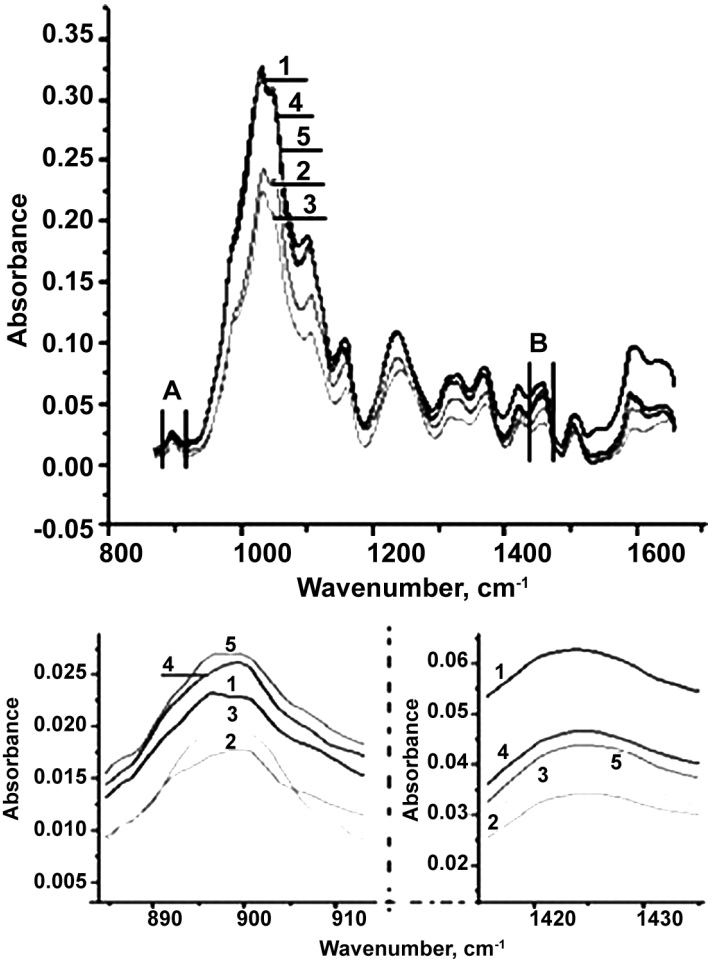

Figure 4. FT-IR spectra of PSF samples: PSF (1), HPSF (2), HPSF-IL1 (3), HPSF-IL2 (4), HPSF-IL3 (5).

values in comparison with those obtained for [BMIM]Cl and [BMP]Cl. The influence of ionic liquid in reaction medium varies in the following order:

$$\text{total crystallinity index (TCI): EMIM]Cl} < \text{[BMIM]Cl} < \text{[BMP]Cl}$$

Table II. Crystallinity indexes and hydrogen bonding intensityfor PSF before and after hydrolysis

Sample	TCI (1372/2900)	LOI (1430/897)	HBI (3308/1330)
PSF	0.52	2.72	4.34
HPSF	0.60	1.97	3.95
HPSF-IL1	0.66	1.85	3.90
HPSF-IL2	0.56	1.62	3.92
HPSF-IL3	0.71	1.60	3.91

lateral order crystallinity index (LOI): [BMP]Cl < [EMIM]Cl < [BMIM]Cl]

Thus, the well-ordered crystalline phase and the degree of intermolecular regularity of cellulose from PSF were affected by the presence of IL in the reaction medium through changing its structure.

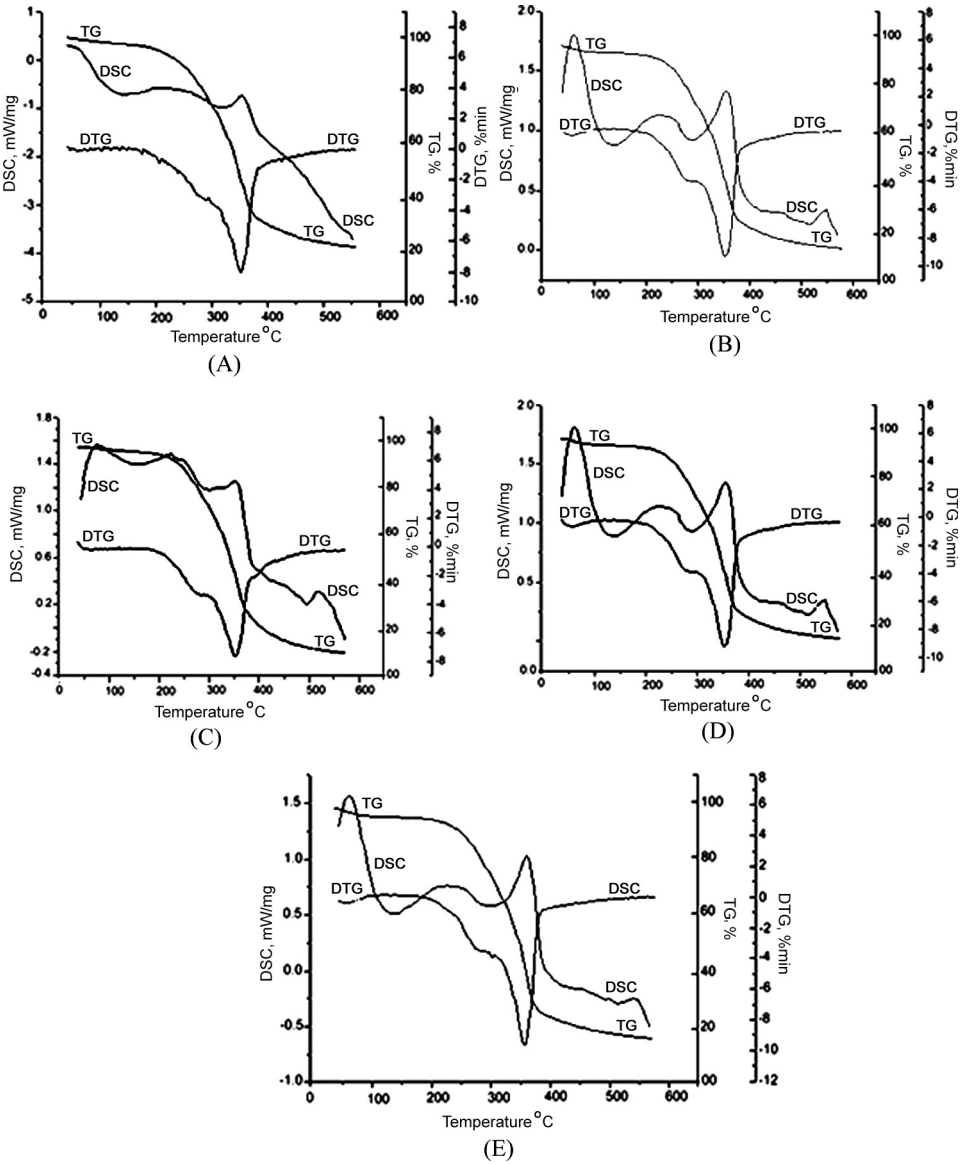

Figure 5. TG-DTG-DSC curves recorded for: PSF (A), HPSF (B), HPSF-IL1 (C), HPSF-IL2 (D), and HPSF-IL3 (E).

Thermal Analysis of Hydrolyzed PSF

Thermograms of the non-hydrolyzed and hydrolyzed samples without ILs and in the presence of ILs are shown in Figure 5. It is well known that the structural organization of polymers influences the shape of thermograms in thermal analysis of cellulose materials.

The mass losses, at around 100°C before the onset temperature, are related to water evaporation. The maximum degradation step at 350°C for PSF (Figure 5A), 360°C for HPSF (Figure 5B), 353°C for HPSF-IL1 (Figure 5C), 352°C for HPSF-IL2 (Figure 5D), and 352°C for HPSF-IL3 (Figure 5E) is assigned to the cellulose degradation.

Under the simultaneous action of enzyme and ionic liquids the degree of structural order organization of cellulose is affected. The lower degradation temperature of PSF hydrolyzed in the presence of IL than that for HPSF (without IL) is attributed to the higher crystalline component percentage in HPSF.

The HPSF sample shows 14.2% residues at 600°C due to charring. Lower values were obtained for HPSF-IL2 and HPSF-IL3. The moisture content that is calculated on the weight loss seen up to 100°C for all hydrolyzed PSF samples was 9–11%. The weight loss observed in the first step degradation for hydrolyzed samples without IL was 86.2% and in the presence of IL it was 85.5% for HPSF-IL1, 89.8% for HPSF-IL2, and 86.5% for HPSF-IL3. This shows that the hydrolysis process in the first step gives an increase of above 4% weight loss for HPSF-IL2 compared to HPSF.

The thermal decomposition of PSF after enzymatic hydrolysis depends on the balance of the two events (decomposition/volatilization of byproducts), both endothermic and exothermic peaks being observed (Figure 5).

At low temperatures, an endothermic process was detected at approximately 60–70°C. The exothermic peaks related to the carbohydrate decomposition appeared near 300°C and 330°C, respectively, and those related to the degradation of lignin appeared from 500°C and higher. The onset temperature of the decomposition (T_d) and the maximum heat flow ($Q_{d\ max}$) with the associated temperature ($T_{d\ max}$) of the HPSF samples under study are presented in Table III.

In this process, higher onset temperatures are associated with higher thermal stability. Based on DSC scans, we can state that HPSF-IL3 is the most thermally stable. The onset temperature of the thermal decomposition process of HPSF-IL1 and HPSF-IL3 is higher than the onset temperatures for HPSF. This behavior could be related to the modification of the ordered structure of samples as a result of the synergetic effect of ionic liquid and enzyme. The higher molecular orientation degree of PSF hydrolyzed in the presence of IL than that of HPSF results in an improvement

Table III. Characteristic parameters from DSC scans recorded for thermal decomposition of HPSF and HPSF-IL

Sample	T_d (°C)	$Q_{d\ max}$ (mW/mg)	$T_{d\ max}$ (°C)
HPSF	265	0.94	295
HPSF-IL1	280	1.16	310
HPSF-IL2	267	0.93	297
HPSF-IL3	285	0.58	315

in the thermal characteristics. The $Q_{d\ max}$ (maximum heat flow) of HPSF-IL3 (Table III) has a lower value due to its improved crystalline structure.

CONCLUSIONS

The present article focuses on the study of hydrolysis using as substrate poplar seed floss (PSF), which is a cellulose-enriched material. This is in agreement with the current tendency to prioritize developing processes under environmentally friendly conditions. Poplar seed floss treated with cellulase and ionic liquid was hydrolyzed more easily than that hydrolyzed without IL.

The hydrolysis rates were accompanied by an increase of the ordered structure of cellulosic materials. Thus, the thermal decomposition of PSF after enzymatic hydrolysis depends on the balance of the two events (decomposition/volatilization of byproducts), both endothermic and exothermic peaks being observed. In this process, the higher values for the onset temperatures should be associated with higher thermal stability.

The presence of ionic liquids in reaction medium seems to improve the hydrolysis rate, although their costs should be significantly reduced and future investigation of this subject is needed.

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