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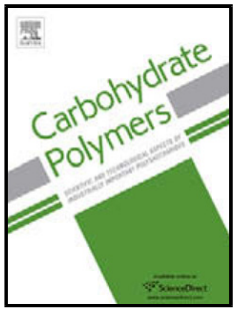

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HYDROLYSIS OF DILUTE ACID-PRETREATED CELLULOSE UNDER MILD HYDROTHERMAL CONDITIONS

by

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Abstract

The hydrolysis of dilute acid-pretreated cellulose was investigated in a conventional oven and under microwave heating. Two acids – sulfuric and oxalic – were studied. For both hydrothermal conditions (oven and microwave) the resultant total organic carbon (TOC) values obtained by the hydrolysis of the cellulose pretreated with sulfuric acid were higher than those obtained by the hydrolysis of the cellulose pretreated with oxalic acid. However, the dicarboxylic acid exhibited higher hydrolytic efficiency towards glucose. The hydrolysis of cellulose was greatly promoted by microwave heating. The Rietveld method was applied to fit the X-ray patterns of the resultant cellulose after hydrolysis. Oxalic acid preferentially removed the amorphous region of the cellulose and left the crystalline region untouched. On the other hand, sulfuric acid treatment decreased the ordering of the cellulose by partially disrupting its crystalline structure.

Keywords: *cellulose, hydrolysis, glucose, dilute acid, microwave assisted*

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1. Introduction

Growing concerns about the depletion of fossil fuels, the impact of CO_2 emissions, and increasing energy demands have encouraged the search for sustainable energy sources. The development of feasible processes for converting cellulosic biomass into valuable chemicals and fuels has received a great deal of attention as a means of replacing non-renewable feedstocks (Gallezot, 2012; Huber, Iborra, & Corma, 2006).

Cellulose is the most abundant and renewable biopolymer in nature (Pu, Ziemer, & Ragauskas, 2006). It needs to be fractionated into its components (monomeric sugars) by hydrolytic processes if its versatility as feedstock is to be maximised (Zhao et al., 2007). Monomeric carbohydrates are the most important materials that can be directly used as feedstock for the production of bio-based chemicals (Srokol et al., 2004). In this regard, the hydrolysis of biomass has been investigated for some considerable time (Sun & Cheng, 2002).

The difficulty of efficiently hydrolysing the recalcitrant crystalline cellulose has proven to be a major barrier to cellulose being extensively used in bio-energy production (Himmel et al., 2007). Cellulose is composed of glucose units linked by β-1,4-glycosidic bonds (or acetal bonds) to create long chains. The linear structure of the cellulose chain forms intra- and intermolecular hydrogen bonds which lead to the chains becoming microfibrils with both crystalline regions and areas of less ordered or amorphous cellulose. The crystal structure and hydrogen bonding in cellulose greatly limit the access of reactants and catalysts to β-1,4-glycosidic bonds. Water is completely excluded from the crystalline regions in cellulose.

The crystallinity of cellulose has been claimed to be one of the most important influences on the kinetics of cellulose hydrolysis. It has been reported that dilute-acid pretreatment of softwood feedstocks increases cellulose crystallinity by preferential

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degradation of the less ordered amorphous cellulose (Sannigrahi, Ragauskas & Miller, 2008). The hydrolysis rate of the amorphous region is much higher than that of the crystalline region in cellulose (Zhao et al., 2006). Although there is now considerable experimental evidence to suggest that the reactivity of cellulose can be increased if its crystallinity is partially reduced, many of the underlying mechanisms and their interplay have not been studied systematically (Van de Vyver, Geboers, Jacobs, & Sels, 2011).

In the hydrolysis of cellulose, appropriate pretreatment is an essential step for effective conversion to fuels and chemicals. Several pretreatment methods have been reported, including biological, physical, chemical and physicochemical methods (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010; Haghighi Mood et al., 2013; Kumar, Barrett, Delwiche, & Stroeve, 2009). A desirable pretreatment should not only provide higher sugar yields and limited amounts of degradation byproducts but also cost less in terms of equipment, energy and catalyst. Chemical pretreatment with dilute sulfuric acid has become a state-of-the-art technology for hydrolysing lignocellulosic biomass. However, inorganic acids need to be neutralized and removed to prevent negative effects on downstream processing. As a result, organic dicarboxylic acids, such as oxalic, maleic and fumaric acids, are appearing as alternatives to enhance cellulose hydrolysis. Additionally, oxalic acid is less toxic to yeasts and other microbes than acetic acid, does not inhibit glycolysis and does not produce noxious odors (Kootstra, Beeftink, Scott, & Sanders, 2009). Among the physicochemical pretreatment methods, the promoting effect of microwave irradiation on dilute alkali- and acid-catalysed hydrolysis of cellulosic materials has recently been reported (Fan et al., 2013; Guo, Fang, Xu, & Smith, 2012; Peng, Chen, Qu, Li & Xu, 2014).

In the present study, we investigate the hydrolysis of cellulose under mild operating conditions, and compare the effect of dilute-acid pretreatment using sulfuric acid and

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oxalic acid, as well as the effect of microwave-assisted pretreatment. Furthermore, we analyse the structural changes in terms of cellulose crystallinity occurring during the hydrolysis process.

2. Experimental

2.1. Materials

Commercially available microcrystalline cellulose powder (Sigma-Aldrich) was used for the hydrolysis tests. The chemicals used include sulfuric acid (1/3 p/v, Panreac), oxalic acid (98%, Sigma-Aldrich), glucose (99%, Panreac) and cellobiose (99%, Panreac). All the chemicals were used without further purification.

2.2. Hydrolysis experiments

Cellulose was pretreated by impregnation with sulfuric acid (SA) or oxalic acid (OA). In order to examine the influence of the acid loading, two different concentrations (3 wt.% and 6 wt.%) were used in dilute-acid conditions. The samples were denoted as SA3, OA3 and SA6, OA6, respectively). The acid-impregnated cellulose samples were dried at 373 K for 20 h.

In all the hydrolysis experiments of the acid-pretreated cellulose samples, 0.3 g of cellulose and 20 mL of water were loaded into a PTFE reactor. The initial pressure of the reactor was atmospheric pressure at room temperature.

Conventional hydrolysis treatment was carried out in an oven, heated at 393 K (or 413 K, for a series of experiments). Reaction time varied from 2 to 4, 6, 8, 12, 16, 20 and 28 h. Additionally, in order to evaluate the effect of microwave irradiation on hydrolysis, microwave-assisted hydrolysis experiments were performed in a microwave system (Milestone Ethos) at 393 K for increasing reaction times of 1, 2, 4 and 6 h.

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After hydrolysis, the liquid fraction (hydrolysate) was separated from the solid by vacuum filtration and analysed by TOC and HPLC. The solid products were washed with 40 mL of water, filtered, dried and kept for further analysis by XRD, NMR and SEM.

2.3. Characterization of liquid products

The concentration of total water soluble organic compounds in the liquid samples after hydrolysis was measured by a Total Organic Carbon (TOC) Analyzer (Analytik jena, Multi N/C 2100). In the case of the samples treated with oxalic acid, the TOC values were corrected by subtracting the nominal carbon content due to the organic acid.

The concentration of glucose and cellobiose in the hydrolysates was determined using an HPLC system (Agilent Technologies, 1100 series) equipped with an ICSep ICE-COREGEL 87H3 column and a refractive index detector (RID). The aqueous mobile phase was deionized and its pH was then controlled to 2.2 by addition of sulfuric acid. Operating conditions for the HPLC column were 313 K with a mobile phase flow rate of 0.6 mL/min. For analysis, 20 μL of sample was injected and complete sample elution was achieved within 30 min. Glucose and cellobiose solutions were used as external standards for quantification. Before analysis all samples were filtered through a 0.45 μm porous filter.

The selectivity to glucose was calculated according to the following equation:

$$Glucose\ selectivity\ (\%) = \frac{Glucose\ concentration\ (mgC/L)}{Total\ organic\ carbon\ concentration\ (mgC/L)}\ x\,100\% \qquad (1)$$

2.4. Characterization of cellulose samples

2.4.1. X-ray Diffraction (XRD)

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The XRD analyses of the materials were recorded using a Siemens D5000 diffractometer (Bragg-Bentano for focusing geometry and vertical θ-θ goniometer) with an angular 2θ-diffraction range between 5º and 90º. The samples were dispersed on a Si (510) sample holder with a cavity that was 0.1 mm deep. The cavity was filled with the same amount of sample to ensure the same baseline for all analyses and sample packaging. The data were collected with an angular step of 0.03º at 5 s per step and sample rotation. Cu Kα radiation (λ=1.54056 Å) was obtained from a copper X-ray tube operated at 40 kV and 30 mA.

The crystallinity index (CI) of cellulose was calculated according to the modified Segal method (Eq. 2):

$$\frac{I_{cel} - I_{AM}}{I_{cel}} x\,100\% \qquad (2)$$

where I_{cel} corresponds to the sum of peak intensities from cellulose that appear in the range 2θ = 10-27º and I_{AM} is the intensity of the amorphous peak (2θ= 18º). It should be noted that this CI refers only to a ratio between diffracted intensities, not to a mass ratio.

All diffractograms were fitted with the TOPAS software (V4.2, Bruker AXS GmbH, Karlsruhe, Germany). This software uses the Rietveld method (Rietveld, 1969) and the Fundamental Parameters Approach (Cheary & Coelho, 1992), which consists of calculating the instrumental contribution to the peak width by describing the different components of the diffractometer.

The contribution of crystallite size to the peak width (τ) was calculated by fitting a Lorentzian and Gaussian function (double-Voigt approach) and applying the modified Scherrer equation (Stokes & Wilson, 1942) to the peak width:

$$\tau = \frac{\lambda}{\beta \sin\theta} \qquad (3)$$

where β is the mean integral breadth and λ is the wavelength used.

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The background was regarded as a straight line with slope. The amorphous part of the sample was assigned to a pseudo-Voigt peak at 2θ = 18º with refinable peak width. The cell parameters for cellulose were refined for each sample.

2.4.2. Solid-state ^{13}C Nuclear Magnetic Resonance (NMR)

For NMR analysis, the solid samples were packed into a 4-mm cylindrical Zirconia MAS rotor. NMR measurements were carried out on an Agilent MERCURY_VX spectrometer operating at frequencies of 400 MHz for ^{13}C in a Bruker double-resonance MAS probe head at spinning speeds of 3-4 kHz. All spectra were recorded with a 35° proton pulse, 5μs contact pulse, 6s recycle delay and 350 scans. The line-fitting analysis of spectra was performed using NUTS™ NMR data-processing software (Acorn NMR Inc.). The CI was determined from the areas of the crystalline and amorphous C4 signals using the following formula:

$$\frac{Area_{87-93\,ppm}}{Area_{80-87\,ppm} + Area_{87-93\,ppm}} x\,100\% \qquad (4)$$

2.4.3. Scanning Electron Microscopy (SEM)

The morphology of the cellulose samples was observed by SEM with a JEOL JSM-35C scanning microscope operated at an acceleration voltage of 15 kV. A small portion of each sample powder was coated on a metallic disk holder and covered with a thin gold layer before SEM analysis.

3. Results and discussion

3.1. Hydrolysis of cellulose

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Figure 1 shows the HPLC chromatograms of the liquid products obtained after the cellulose hydrolysis at 393 K under different hydrothermal conditions. Figure 1A and Figure 1B illustrate the product distribution of the cellulose hydrolysis under conventional hydrothermal treatment in an oven and under microwave-assisted hydrolysis treatment, respectively.

The HPLC chromatograms of Figure 1A show the product distribution after 28 h of hydrolysis of the cellulose samples SA3, SA6, AO3 and AO6 in the oven. Figure 1B illustrates the HPLC product distribution after 6 h of microwave-assisted hydrolysis. The main products identified in the resulting hydrolysis solution were aqueous oligomers such as cellobiose (7.8 min), monomers (e. g. glucose) and other water soluble organic compounds (WSOCs). The retention time of glucose under our analytical conditions was about 9 min (see Figure 1).

The results of the HPLC chromatograms of the products (figure 1) show that the glucose concentration is higher in the microwave-assisted hydrolysis for the cellulose pretreated with both sulfuric and oxalic acid. Microwave irradiation dissolves cellulose by disrupting hydrogen bonds between the molecules (Zhang & Zhao, 2009). The presence of other water soluble organic compounds (WSOCs) was more evident when the cellulose was treated with sulfuric acid particularly after the microwave-assisted hydrolysis. On the other hand, the pretreatment of cellulose with oxalic acid favoured the hydrolysis of cellulose to glucose. Oxalic acid is among the strongest organic acids known, and its dicarboxylic properties are thought to provide greater selectivity for the hydrolysis of cellulose (Mosier, Sarikaya, Ladisch, & Ladisch, 2001). For further clarification of these points, see the product distribution during cellulose hydrolysis in Figures 2 and 3.

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Figure 2 illustrates the product distribution during hydrolysis in the conventional hydrothermal treatment in an oven of the cellulose pre-treated with the different concentrations of sulfuric acid (Figures 2A and 2B) and oxalic acid (Figures 2C and 2D). Figure 3 shows the product distribution of the microwave-assisted hydrolysis of the cellulose pretreated with sulfuric acid (Figure 3A and 3B) and oxalic acid (Figure 3C and 3D).

In the conventional hydrothermal process (in an oven) the product distribution of the cellulose hydrolysis was monitored for 28 h with samples being taken at different times (see Figure 2). Figure 2 shows that the hydrolysis of the cellulose treated with the different diluted acids reached a plateau of glucose concentration after about 20 h of hydrothermal treatment. In the case of cellulose sample SA3 the glucose concentration after 20 h of hydrolysis was about 125 mg/L (Figure 2A) whereas for the cellulose treated with 6 wt.% of sulfuric acid (SA6) it was about 95 mg/L (Figure 2B). It seems that a more concentrated sulfuric acid treatment generated more degradation products. Sample SA6 promotes glucose degradation (Figure 2B) more than sample SA3 (Figure 2A). In the case of the cellulose pre-treated with 6 wt.% of sulfuric acid (SA6) the total water soluble organic compounds (TOC) was about 350 mg/L, which was close to the TOC value for the hydrolysis of the cellulose pretreated with 3 wt.% of sulfuric acid (SA3). In Figure 2, the TOC values for the hydrolysis of the cellulose pretreated with sulfuric acid were always higher than those for the hydrolysis of the cellulose treated with oxalic acid.

The results of the oven hydrolysis of the cellulose pretreated with oxalic acid (OA3 and OA6) are represented in Figures 2C and 2D, respectively. In particular, note the difference in the hydrolysis of cellulose to glucose between sample OA6 (Figure 2D) and SA6 (Figure 2B). Figure 2D shows that the resultant concentration of glucose after

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20 h of cellulose hydrolysis was about 200 mg/L. It is also important to notice that the resultant concentration of the WSOCs in the hydrolysis of the cellulose treated with oxalic acid was in all cases lower than in the case of the cellulose treated with sulfuric acid. For instance, for the cellulose treated with 6 wt.% of oxalic acid (Figure 2D) the concentration of WSOCs was about 25 mg/L whereas for the cellulose treated with 6 wt.% of sulfuric acid it was about 200 mg/L (Figure 2B). It is also noted that the cellobiose concentration was always higher in the products resulting from the conventional hydrothermal conditions (Figure 2) than from microwave-assisted hydrolysis (Figure 3).

The results indicate that there is an important difference between the two acids used (sulfuric and oxalic acids) in the resultant hydrolysis. The dicarboxylic acid exhibited higher hydrolytic efficiency towards glucose. This hydrolytic efficiency presented an even more significant difference when the hydrolysis of cellulose was assisted by microwave irradiation (see Figure 3). In the microwave-assisted hydrolysis the product distribution was monitored for 6 h. Figure 3 shows the difference between the TOC in solutions resulting from the microwave-assisted hydrolysis of cellulose and the conventional oven hydrolysis (see Figure 2). With respect to the cellulose treated with 6 wt.% of sulfuric acid it should be pointed out that its microwave-assisted hydrolysis (Figure 3B) produced a resultant TOC of about 900 mg/L after 6 h. On the other hand, the conventional oven hydrolysis of the cellulose treated with 6 wt.% of sulfuric acid produced a cellulose dissolution of about 300 mg/L after 28 h (Figure 2B). Again the microwave-assisted hydrolysis of the cellulose treated with oxalic acid (Figures 3C and 3D) favoured the hydrolysis of cellulose to glucose. This effect is more remarkable when the resultant hydrolysis of the cellulose treated with the dicarboxylic acid (Figures 3C and 3D) is compared to the corresponding resultant hydrolysis of the cellulose

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treated with sulfuric acid (see Figures 3A and 3B). In the case of cellulose treated with 6 wt.% of oxalic acid the resultant concentration of glucose after 6 h of hydrolysis was about 425 mg/L (Figure 3D) versus 275 mg/L of glucose from the hydrolysis of the cellulose treated with 6 wt.% of sulfuric acid (Figure 3B). Moreover, based on the results of Figure 3, the resultant TOC values of the hydrolysis of the cellulose pretreated with sulfuric acid were higher than the TOC values obtained for the hydrolysis of the cellulose pre-treated with oxalic acid. For instance, a TOC of 900 mg/L was obtained for cellulose sample SA6 (Figure 3B) whereas a TOC of 525 mg/L was obtained for the cellulose sample OA6 (see Figure 3D).

Figure 4 provides a brief summary of the hydrolysis of cellulose pre-treated with the different diluted acids and in different hydrolysis conditions. It presents the resultant concentration of the total water soluble organic compounds (TOC) (Figure 4A) and the selectivity to glucose (Figure 4B) after 6 h of hydrolysis. Two different temperatures for the conventional oven are depicted: 393 K and 413 K. Figure 4A clearly shows that in both hydrothermal conditions (oven and microwave) the resultant TOC values obtained after the hydrolysis of the cellulose pretreated with sulfuric acid were always higher than those obtained after the hydrolysis of the cellulose pre-treated with oxalic acid. However, Figure 4B shows that the selectivity to glucose after the hydrolysis of cellulose treated with oxalic acid in both the hydrothermal conditions was always higher than in the corresponding cellulose sample treated with sulfuric acid. For instance, at 393 K the selectivity after 6 hours of microwave-assisted hydrolysis of cellulose pre-treated with 6 wt.% of oxalic acid was about 85% whereas for the cellulose treated with 6 wt.% of sulfuric acid the glucose selectivity was about 30%. The hydrolysis of the cellulose pretreated with 6 wt.% of oxalic acid in an oven at 413 K presented a glucose selectivity of about 65%. The microwave heat enhances hydrolysis performance. These

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findings reveal that the hydrolytic efficiency of the dicarboxylic acid toward glucose is much higher than that of the cellulose treated with sulfuric acid. Previous studies have also shown that dicarboxylic organic acids can hidrolyze β-1,4-glycosidic bonds more selectively than sulfuric acid (Mosier, Ladisch, & Ladisch, 2002). It is also known that cellulytic enzymes catalyze hydrolysis through a general acid-base mechanism mediated by two carboxylic acids. The oxalic acid with its two pKa´s might mimic this reaction through similar ion-pair mechanisms (Lu & Mosier, 2007). This may result in a more effective attack on the β-1,4 linkage while the fact that its ionization potential is weaker than that of sulfuric acid may reduce subsequent degradation products, which would explain the lower concentration of WSOCs observed in the hydrolysis of cellulose pretreated with oxalic acid.

3.2. Characterization of cellulose after hydrolysis

The structural modification of cellulose by hydrolysis of the pretreated samples was investigated using several techniques: X-ray Diffraction (XRD), ^{13}C Nuclear Magnetic Resonance (NMR) and Scanning Electron Microscopy (SEM). The main results obtained by these characterization methods are summarized below.

3.2.1. XRD

The features of the cellulose samples recovered after hydrolysis at the longest reaction times studied (28 h for conventional hydrolysis experiments conducted in an oven and 6 h for microwave-assisted hydrolysis) were examined using XRD and compared to untreated cellulose. Figure 5 shows the XRD patterns of the cellulose samples following acid pretreatment and hydrolysis in the conventional oven (Figure 5A) and the microwave oven (Figure 5B).

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Due to the fact that all diffractograms were very similar, the diffractograms had to be fitted in such a way that both the integrated intensity of the crystalline part and the amorphous part were not induced by the operator. In this regard, the use of the crystal structure in the refinement process gives the theoretical intensity of all the peaks that belong to this phase. In the case of cellulose, there are so many overlapping peaks that a common error is to assume that in the range 2θ= 10-27º there are only 4 peaks at ≈14.7, 16.4, 20.3 and 22.6º whereas with the crystal structure there are 11 peaks. The cellulose polymorph that was present in all samples was identified as the Cellulose-Iβ and the crystal structure was taken from (Nishiyama, Langan, & Chanzy, 2002) ($P2_1$, a: 7.784(8) Å, b: 8.201(8) Å, c: 10.380(10) Å, γ: 96.5º). Near the peak assumed to correspond to the "amorphous part" (at 2θ= 18º), cellulose exhibits the (111) diffraction peak such that if the crystal structure is not considered the amorphous content may be overestimated.

Figure 6 shows the good agreement between the experimental diffractogram and the one calculated from the crystal structure of cellulose sample SA6 after 28 h of hydrolysis in an oven and cellulose sample OA6 after 6h of hydrolysis in a microwave.

The crystallinity index (CI) for all samples, as well as for the original cellulose, was calculated from the XRD data and the results are summarized in Table 1. The CI of the starting cellulose material is relatively high (CI = 78.5%) and, after the hydrolysis of the pretreated samples, there was an opposite change in the CI, depending on the acid used. The results obtained from the samples treated with oxalic acid show a slight increase in CI values (around 80-81%) in comparison with the original cellulose. This increase in the degree of crystallinity suggests that there was a preferential attack by oxalic acid on the amorphous cellulose fraction. On the other hand, the cellulose CI decreased in the samples treated with sulfuric acid, which indicates that the use of sulfuric acid together

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with the pretreatment and the hydrolysis conditions in this study were capable of decreasing the ordering of cellulose by partially disrupting its crystalline structure.

For a given acid and concentration, the use of microwave-assisted hydrolysis instead of conventional hydrolysis did not significantly alter the crystallinity of the residual cellulose.

It can be seen that increasing the amount of sulfuric acid (from 3 to 6 wt.%) decreases the crystallinity index (from 68.6 to 59.9%, for hydrolysis in the oven, and from 74.8 to 59.0%, for hydrolysis in the microwave). However, no significant changes were observed in CI as a result of changing the content of oxalic acid.

3.2.2. NMR

NMR analyses of selected samples were performed to confirm the trend in the CI observed from the XRD data.

Figure 7 shows the ^{13}C NMR spectra of the original cellulose and the samples treated with sulfuric and oxalic acid (both with 6 wt.% acid content and hydrolysed in a microwave oven).

In general, all the samples presented a similar spectrum. The peaks at 61.9 and 64.8 ppm are assigned to the C-6 glucopyranosyl repeating units in cellulose (61.9 ppm for amorphous cellulose and 64.8 ppm for crystalline cellulose). The cluster of resonances around the peaks at 72.2 and 75.8 ppm are assigned to C-2, C-3 and C-5. The peaks at 84.4 and 89.0 ppm are attributed to C-4 (amorphous and crystalline, respectively) and the absorption peak at 105.0 ppm is assigned to C-1 of glucose in cellulose (Wickholm, Larsson, & Iversen, 1998).

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Cellulose CI was determined from the crystalline and amorphous signal areas of the C-4 region. The cellulose crystallinity index of untreated cellulose was determined to be 66.6%. After the hydrolysis of sulfuric acid-pretreated cellulose, the CI decreased to 57.1%, while the CI value of oxalic acid-treated cellulose increased to 70.2%. (All CI values obtained from NMR data are found in Table 2).

These changes in the CI are in good agreement with the variations observed in CI values calculated from the XRD data.

3.2.3. SEM

The SEM micrographs in Figure 8 show the surface of raw cellulose (Figure 8A) and the resultant cellulose samples SA3 (Figure 8B) and SA6 (Figure 8C) after 28 h of hydrolysis in an oven. Figure 8D shows the resultant cellulose sample AO6 after 6 h of hydrolysis under microwave irradiation.

The untreated cellulose sample can be seen to have a smooth surface, whereas the cellulose that was left after hydrolysis presents clear shrinkage folds on the surface. No significant changes were observed on the surface of cellulose samples as a result of the different treatments, the acid used or the concentration of acid.

4. Conclusions

The effects of the type of acid and the hydrothermal treatment on cellulose hydrolysis were investigated. Two acids were used: sulfuric and oxalic acid. The cellulose was pretreated by impregnation with two different concentrations (3 wt.% and 6 wt.%) of each acid. After pretreatment with diluted acid, the cellulose was further submitted to hydrolysis under conventional oven and microwave irradiation. The hydrolysis was considerably enhanced by the use of microwave. The microwave-assisted hydrolysis of

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the cellulose treated with 6 wt.% of sulfuric acid produced a resultant TOC of about 900 mg/L after 6 h, whereas the corresponding hydrolysis in an oven produced a TOC of about 300 mg/L.

The present work studied the effect of reaction conditions such as the type of acid, temperature, reaction time and the use of oven and microwave irradiation during the hydrolysis process. The organic and mineral acids function as catalyst and attack cellulose chains to produce oligomers with varying degrees of polymerization. The oligomers are further hydrolyzed into monomeric sugars (Lee, Iyer & Torget, 1999). To increase the contact area between catalysts and substrates and to increase glucose yield an auxiliary technique such as microwave irradiation during the cellulose hydrolysis have been proposed. Microwave heating may improve the effective contact between acid and solid substrate (Liao et al., 2009). The dilute acid treatment with sulfuric acid was able to partially break down the crystal structure of cellulose whereas the oxalic acid attacked exclusively the amorphous phase. Dicarboxylic acids such as oxalic acid are an alternative to sulfuric acid for cellulose pretreatment. Indeed as noticed in the present work the dicarboxylic acid exhibits a higher catalytic efficiency to the glucose formation than sulfuric acid when applied under the same hydrolysis conditions. Characterization by the Rietveld method and the NMR data are in agreement in terms of the crystalline index. Pretreating the cellulose with oxalic acid also seems to selectively remove the amorphous region of the cellulose and leave the crystalline region untouched as was observed by the characterization of the remaining cellulose after the hydrolysis.

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Figure captions

Figure 1. HPLC chromatograms of selected liquid samples after hydrolysis in A) oven and B) microwave.

Figure 2. Changes in aqueous product concentration (mg/L) versus reaction time during cellulose hydrolysis in a conventional hydrothermal treatment (oven) at 393 K. Cellulose samples pretreated with A) 3 wt.% of sulfuric acid (SA3), B) 6 wt.% of sulfuric acid (SA6), C) 3 wt.% of oxalic acid (OA3) and D) 6 wt.% of oxalic acid (OA6).

Figure 3. Changes in aqueous product concentration (mg/L) versus reaction time during cellulose hydrolysis assisted by microwave irradiation at 393 K. Cellulose samples pretreated with A) 3 wt.% of sulfuric acid (SA3), B) 6 wt.% of sulfuric acid (SA6), C) 3 wt.% of oxalic acid (OA3) and D) 6 wt.% of oxalic acid (OA6).

Figure 4. A) TOC values for cellulose hydrolysis in different hydrothermal conditions after 6 h of hydrolysis. B) Glucose selectivity obtained from cellulose hydrolysis after 6 h.

Figure 5. X-ray diffractograms of selected solid samples after hydrolysis in A) oven and B) microwave.

Figure 6. XRD – Calculated and observed X-ray diffractograms for samples AS6 and AO6 after 28 h of hydrolysis in a conventional oven showing the peak position for the amorphous part.

Figure 7. NMR spectra of selected cellulose samples.

Figure 8. SEM micrographs of A) raw cellulose and solid samples after hydrolysis, B) cellulose pretreated with 3 wt.% of sulfuric acid after 28 h of hydrolysis in oven, C) cellulose pretreated with 6 wt.% of sulfuric acid after 28 h of hydrolysis in oven and D) cellulose pretreated with 6 wt.% of oxalic acid after 6 h of hydrolysis in microwave.

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Table 1. CI from XRD data of recovered cellulose samples after hydrolysis at the longest reaction times studied (28 h for conventional hydrolysis experiments conducted in an oven and 6 h for microwave-assisted hydrolysis)

Sample	CI (%)	Crystallite size (nm)
Cellulose	78.5	6.0(2)
SA3 – Oven	68.6	6.0(2)
SA6 – Oven	59.9	6.3(2)
SA3 – MW	74.8	5.7(1)
SA6 – MW	59.0	6.2(2)
OA3 – Oven	79.8	6.0(1)
OA6 – Oven	81.3	5.9(1)
OA3 – MW	80.4	6.1(1)
OA6 – MW	81.1	5.9(1)

Table 2. CI from NMR data

Sample	CI (%)
Cellulose	66.6
SA6 – MW	57.1
OA6 – MW	70.2

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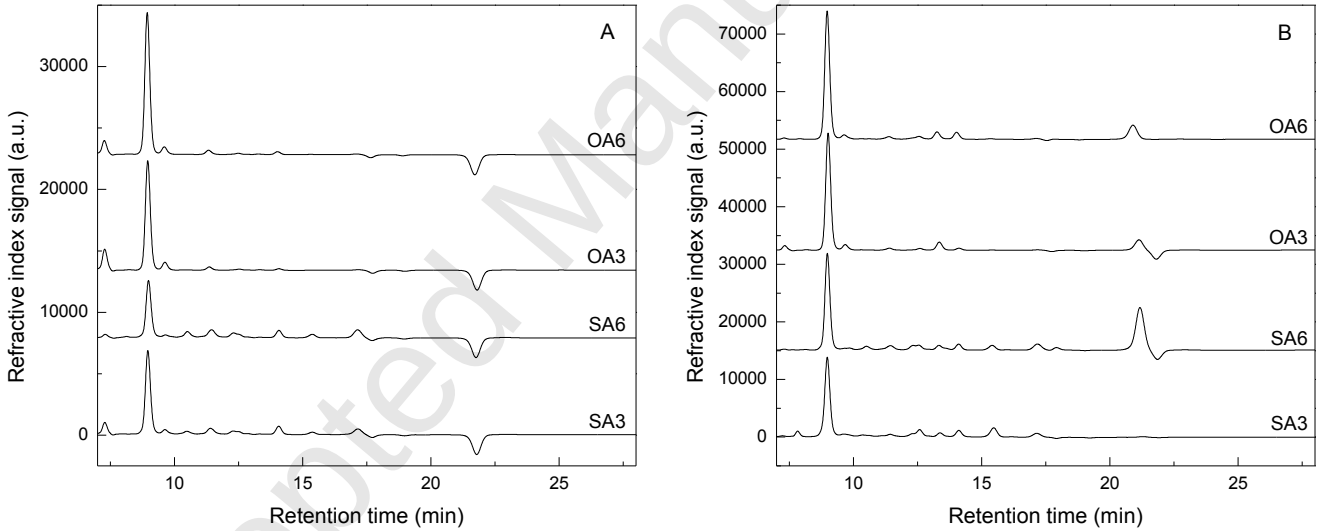
Figure 1



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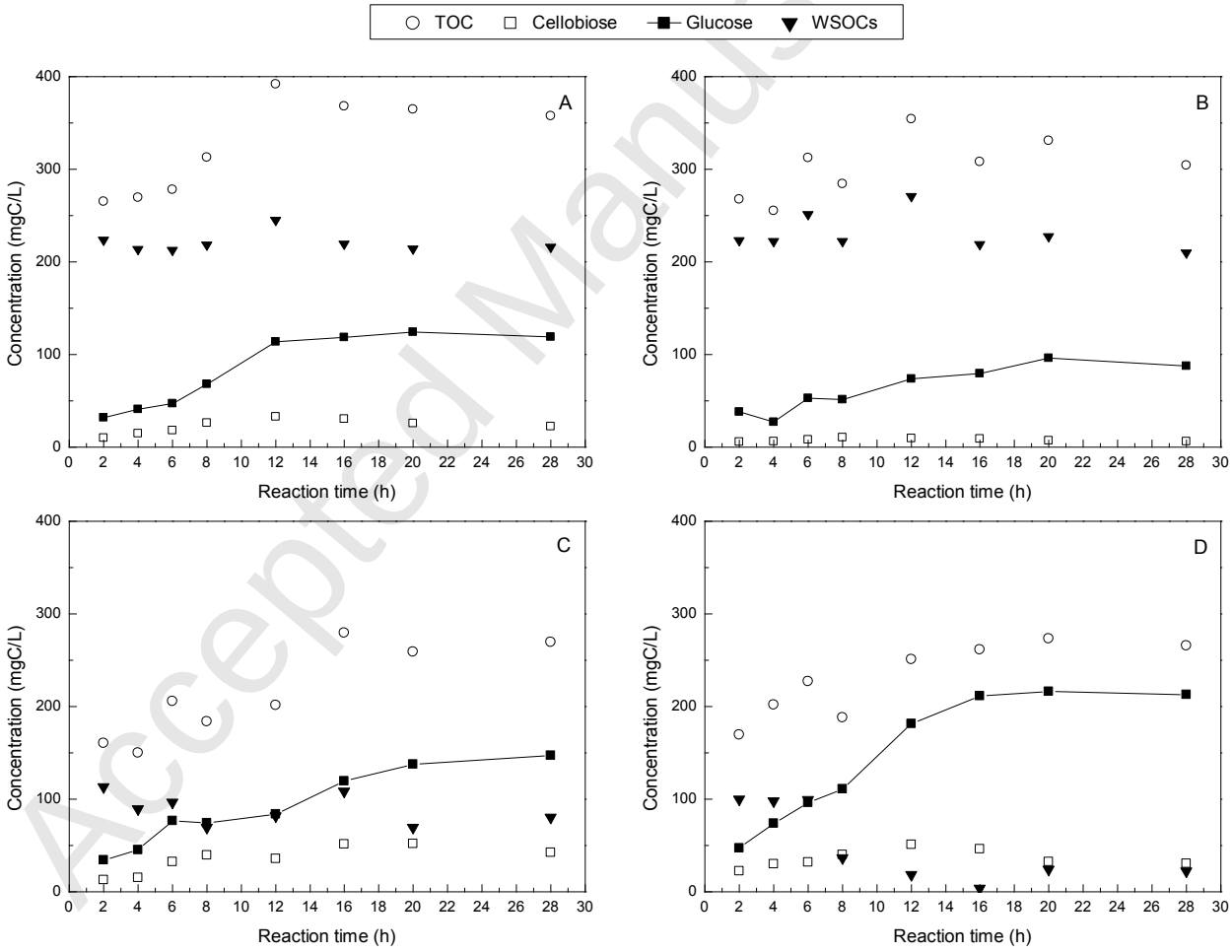

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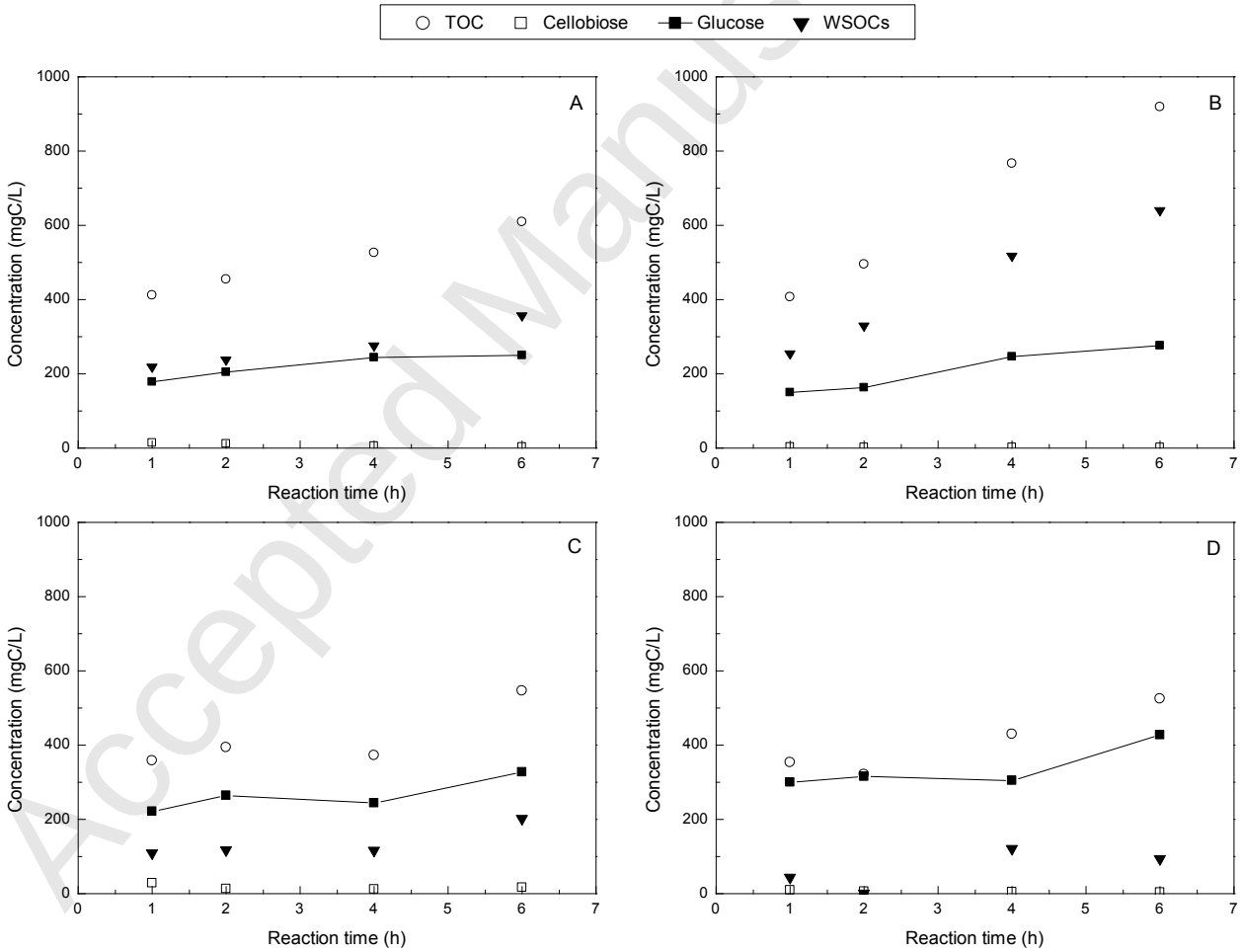

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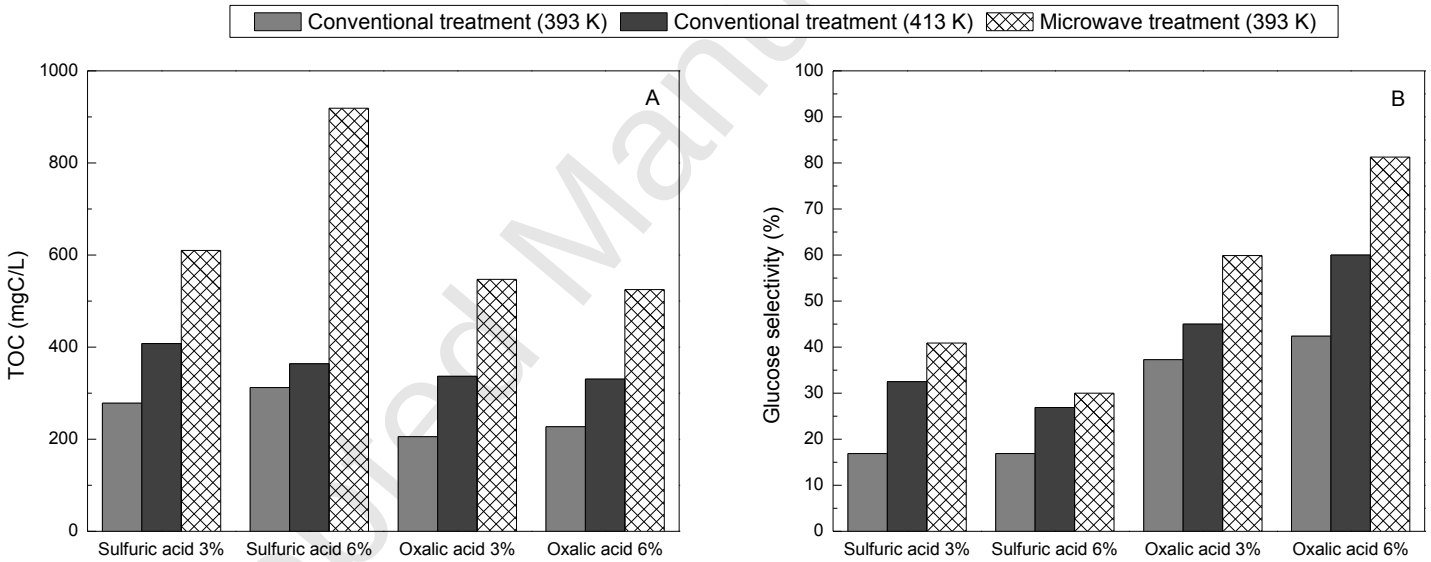

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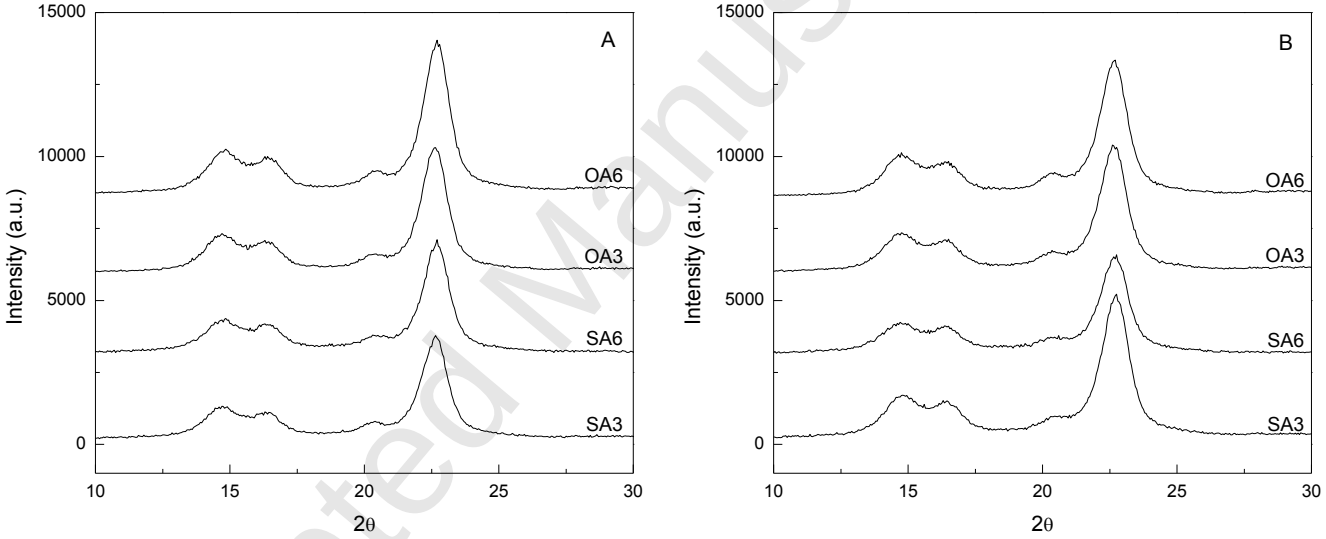

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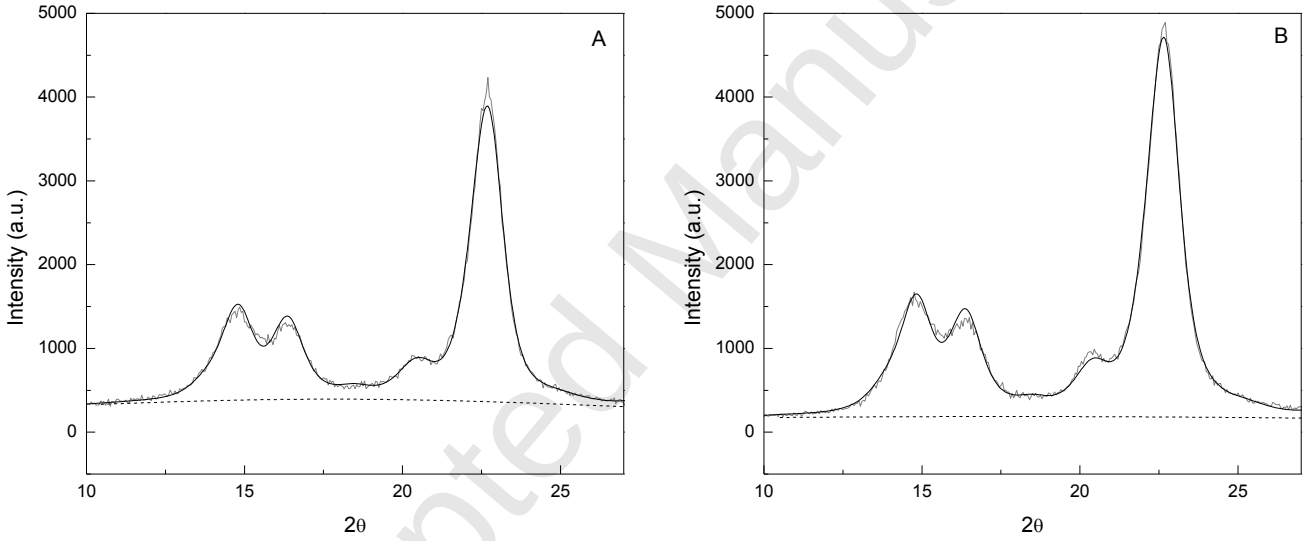

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Figure 7

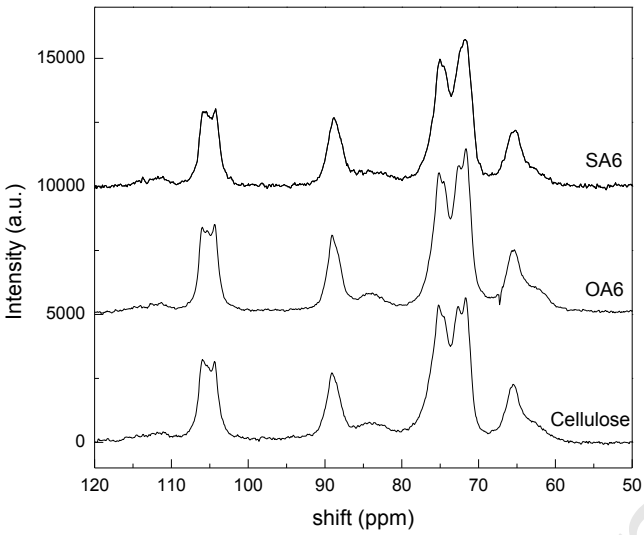

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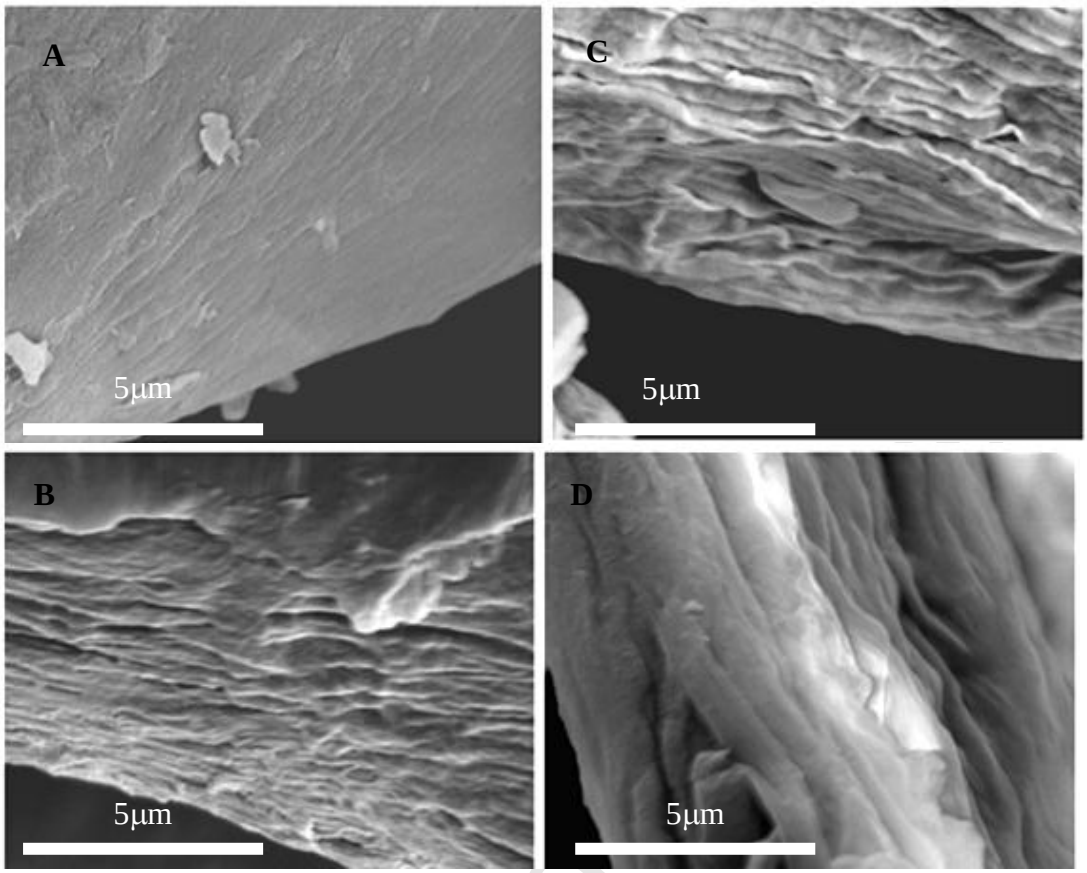
Figure 8



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Research highlights

♦The hydrolysis of cellulose assisted by microwave irradiation was greatly promoted. ♦The selectivity to glucose was enhanced by the dicarboxylic acid. ♦ Oxalic acid preferentially removed the amorphous region of the cellulose.

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