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Nanocrystalline cellulose extraction process and utilization of the byproduct for biofuels production

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ABSTRACT

Cellulose consists of amorphous and crystalline regions. It is the crystalline regions which may be exploited to produce nanocrystalline cellulose (NCC). In order to extract nanocrystalline cellulose from native cellulose, sulfuric acid hydrolysis is typically used. The amorphous regions of cellulose are hydrolyzed and degraded into soluble products while the crystalline regions remain intact. In an effort to make the NCC extraction process more feasible, a new process was developed to recover and utilize the hydrolyzed regions of cellulose as a byproduct. The acid hydrolyzed amorphous regions were separated and then recovered (regenerated) into solid particles. XRD data revealed that the recovered material is characteristic of cellulose II. Hydrolysis conditions were optimized to maximize the yield of the recovered material and at the same time produce NCC material. Preliminary experiments showed yield values of approximately 61% for the cellulose I crystalline portions and values of about 21.7% for the recovered material (cellulose II). Enzymatic hydrolysis experiments of the recovered material revealed high susceptibility to enzymatic hydrolysis which makes it a promising source for biofuels production.

1. Introduction

Cellulose is the most abundant and renewable biopolymer on earth and is obtained from renewable resources such as biomass (Aygan & Arikan, 2008). Cellulose, like mostpolymers, consists of crystalline and amorphous regions. Nanocrystalline cellulose (NCC) or cellulose nanocrystalshave high strength due to their dense and orderly crystalline structure. The modulus of elasticity of the perfect crystal of native cellulose has been calculated by many authors and estimated to be between 130 and 250 GPa and the tensile strength is assessed to be approximately between 0.8 and 10 GPa (Zimmermann, Pöhler, & Geiger, 2004). Other advantages include the fact that cellulose crystals are not costly and that they are light weight with nanoscale dimensions and unique morphologies (Habibi, Lucia, & Rojas, 2010). All these factors, along with their tremendous surface area, make NCC an ideal reinforcing material for use in the fabrication of composites, which may be used in various applications such as packaging. NCC may also be employed in the production of bionanocomposites such as those with the potential to be used in tissue engineering.

It is reported that the amorphous regions of cellulose are more susceptible to acid attack than its crystalline regions (Siqueira, Bras, & Dufresne, 2010). Many methods have been investigated to extract the crystalline regions of cellulose. Cellulose nanocrystal suspensions were initially introduced in the late 40s (Rånby, Banderet, & Sillén, 1949). These initial findings were corroborated by a number of studies and cellulose nanocrystals became much more well-known in the early 90s (Revol et al., 1994). In fact, defect-free, rod-like crystalline residues can be obtained as a result of subjecting cellulose fibers to acid hydrolysis (Habibi et al., 2010). Consequently, in order to separate the NCC, acid hydrolysis must be carried out in controlled conditions to ensure that only the amorphous segments of the cellulose are acted on, leaving the crystalline segments intact. Parameters that must be carefully monitored are the hydrolysis time and temperature as well as the concentration of the acid used (Siqueira et al., 2010). Sulfuric acid and hydrochloric acid are most commonly considered for the acid hydrolysis of cellulose due to their high strength which is essential to hydrolyze the glucosedic bond found in cellulose. At the same time, sulfuric acid tends to be preferred as it provides more stable aqueous suspensions than hydrochloric acid (Siqueira et al., 2010). This is because it leads to the formation of sulfate groups on the surface of the cellulose (Durán, Lemes, Durán, Freer, & Baeza, 2011).

The selection of the acid concentration is a major factor considered when targeting the amorphous regions of cellulose during the dissolution process. Previous work reported that an acid concentration of 64% can effectively dissolve the amorphous portion with small effect on the crystalline portion (Revol et al., 1994). In fact, the concentration of sulfuric acid, in hydrolysis reactions to obtain NCC, does not vary much from a typical value of ca. 65% (Habibi et al., 2010).

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Cellulose has a supramolecular structure. As a result of acid hydrolysis, the structure of the cellulose is broken down to a certain degree, giving lower molecular weight constituents (Iranmahboob, Nadim, & Monemi, 2002) and yielding a molecular weight distribution consisting of smaller molecular fractions (Lin, Chang, & Hsu, 2009). In addition, Xiang, Lee, Pettersson, and Torget (2003) have discussed the molecular mechanism of the acid hydrolysis of cellulose, proving how the "cleavage of the β-1–4-glycosidic bond" leads to hydrolyzed products of smaller molecular weights. Moreover, the hydrolysis of native cellulose leads to a decrease in its degree of polymerization (DP) (Habibi et al., 2010).

Bioethanol is a renewable fuel which has much potential to be used in the transportation sector in the near future. In fact, it is already used on a commercial scale in places like Brazil and the United States (Galbe & Zacchi, 2007). Current sources of bioethanol are "starch and sugar crops" but they have the drawback of being sources of food as well (Zheng, Pan, & Zhang, 2009). An important alternative source of bioethanol is therefore cellulose. The process most often considered for conversion of cellulose to fuel is enzymatic hydrolysis. It has the advantages of "higher yields, minimal byproduct formation, low energy requirements, mild operating conditions, and environmentally friendly processing" (Zheng et al., 2009). In addition, it is a highly specific process which is less expensive than acid or alkaline hydrolysis since enzymatic hydrolysis is usually carried out under mild conditions (Sun & Cheng, 2002). Moreover, enzymatic hydrolysis has the benefits of being a low-energy intensive process and being able to produce sugars in large quantities with actual yield values that closely match the theoretically calculated values (Abushammala, 2011).

Cellulases, which usually consist of multiple enzymes, are used as part of enzymatic hydrolysis to degradate cellulose (Takahashi, 2007). They can be obtained from both bacteria and fungi, but fungi, specifically Trichoderma, are preferred as their growth rates are faster and they do not require anaerobic conditions (Sun & Cheng, 2002).

The structure of cellulose as well as its insolubility (van Wyk, 1997) make it difficult for the enzymes to work on it during the hydrolysis process. In fact, it is intrinsically resistant to enzyme attack (Pan, Xie, Gilkes, Gregg, & Saddler, 2005). Its crystalline regions are such that they cannot be easily penetrated by water or enzymes (Florian, Kronkright, & Norton, 1990) and the individual cellulose fibrils may possibly aggregate together, leading to a greater decrease in the surface area available for the enzymes to act on. As a result, pretreatment of the cellulose becomes a requirement if "reasonable rates and yields" are to be achieved. In general, costly pretreatment procedures are needed to make this possible (Ballesteros, Oliva, Negro, Manzanares, & Ballesteros, 2002). In addition, the enzymes available tend to take "several days to achieve good results" (Badger, 2002).

The factors on which the rate of enzymatic hydrolysis depends are mainly the reaction conditions such as the temperature and pH at which the reaction takes place. What is more, though the cost of the sources of cellulose used in the production of bioethanol is very low, the cost of using these materials to produce sugars which may be fermented into bioethanol "has historically been far too high to attract industrial interest" (Galbe & Zacchi, 2002). Nevertheless, according to a recent report by the United States Department of Energy, "enzyme cost for cellulosic bioethanol is about $0.68 per liter, while the target should be $0.23–0.34 per liter" (Durán et al., 2011). This is due to the efforts of the Novozymes company, which has received contracts from the United States Department of Energy to achieve greater efficiency in the enzymatic hydrolysis process and make it more cost-effective (Durán et al., 2011). Thus much work is being done to make the conversion of cellulose into biofuel a more feasible option.

In this work we present a modified process to produce NCC material and, at the same time, co-produce and recover a new form of cellulose. The recovered cellulose has a fairly open structure, making it more susceptible to enzymatic hydrolysis and as a result, making it a potential material for biofuels production since it can be used more efficiently in enzymatic hydrolysis. Thus it is automatically pretreated as a result of the acid hydrolysis it undergoes. In addition, it is a byproduct of the acid hydrolysis used to produce NCC and is thus usually disposed of. This research shows how this byproduct may be effectively used as an energy source.

2. Materials and methods

2.1. Materials

Cotton cellulose (filter paper) purchased from Whatman® was grinded and used as a source of cellulose. Sulfuric acid, 95–97%, reagent grade, was purchased from Scharlau; ethanol and cellulase from Trichoderma Reesei (10KU); and sodium acetate and acetic acid 100% were purchased from Sigma–Aldrich. Sodium hydroxide was purchased from Riedel-de Haen.

2.2. Preparation of nanocrystalline cellulose (NCC) and acid-soluble cellulose

2.2.1. Cellulose dissolution

10 g of cellulose was mixed with 91 mL of 64%/65%/66% (w/w) H_2SO_4 using the Varian Dissolution System (VK7010) at 45 °C or 23 °C/25 °C with 250 rpm agitation. After the hydrolysis time had elapsed (10–300 min, depending on the hydrolysis trial), 187 mL of a lower concentration (50%) H_2SO_4 was added. The relative amount of cellulose used and the volumes of sulfuric acid involved in these experiments have been taken from the patent by Hashaikeh, Hu, and Berry (2010).

2.2.2. Cellulose centrifugation

The cellulose centrifugation was a two stage process. In the first stage, immediately following the hydrolysis, the cellulose suspension was centrifuged one time at 23 °C using the Allegra™ 25R Centrifuge. The duration and speed of the centrifugation were ten minutes and 4700 rpm respectively. After this, two layers were obtained as a product of the centrifugation, the NCC and the recovered cellulose layers, which were each diluted to approximately four times their original volume, using chilled ethanol for the recovered cellulose layer and chilled water for the NCC layer. They were then left to settle for about 24 h so that a top liquid portion and a bottom solid portion were obtained in each case. Much of the top portion liquid was decanted for both layers which was followed by the second stage of the centrifugation for the bottom solid portion of each layer. The centrifugation conditions were identical to those used in the first stage of centrifugation with the exception that the centrifugation was performed three times for each layer. The solid material of each layer was collected at the end of the centrifugation runs and was dialyzed for three days until the pH was neutralized. The resultant suspensions were weighed and then sonicated using the Hieschler Ultrasonic Processor UP400S for 2–7 min. After dialysis, the yield was calculated by withdrawing a known, small amount of the sample and obtaining its oven-dried weight with the help of the New Brunswick Scientific Innova 40 Incubator Shaker. The yield was calculated based on the solid product weight after drying, compared to the starting weight. The samples were also freeze-dried using the VirTis Wizard 2.0 Freeze Drier.

2.3. Enzymatic hydrolysis

About 100 mg of freeze-dried cellulose was put into a vial that contained 10 mL of 1 M acetate buffer pH 5.13. It was then heated up to 50 °C for 30 min with the help of the New Brunswick Scientific Innova 40 Incubator Shaker before 20 mg of cellulase enzyme was added to it. 0.25 mL samples were withdrawn after 1, 2, 3, 4, 5, 6, 12, 24, and 48 h and mixed with 1.67 mL of 0.08 M NaOH to terminate the process. The glucose concentration was measured for all samples using the YSI 7100MBS bioanalytical system from YSI Life Sciences. The YSI 7100MBS achieves this by taking a sample of size 10–50 μL (Labcompare, 2012). To measure the glucose level, one or more enzymes are immobilized between a polycarbonate and a cellulose acetate membrane. The glucose reacts with the enzyme(s) and the hydrogen peroxide thus formed passes through the cellulose acetate membrane and is then oxidized at a platinum anode. The current which is produced as a result is proportional to the concentration of the glucose (Sun, 2012).

2.4. Microstructure analysis

X-ray diffractograms (XRD) data of the starting material as well as of the oven-dried samples of the hydrolysis trials were obtained with an X-ray diffractometer (PANalytical, X'Pert Pro). The XRD graphs thus obtained were used to find the Crystallinity Index of the NCC produced. This was achieved with the help of the Segal method in which the following formula is used (Thygesen, Oddershede, Lilholt, Thomsen, & Ståhl, 2005):

$$\text{Crystallinity Index} = \left| \frac{I_c - I_A}{I_c} \right|$$

I_c represents the intensity of the principal cellulose I peak at the position of $2\theta = 22.70^\circ$ and I_A represents the intensity of the amorphous cellulose at the position of $2\theta = 18.0^\circ$ (Parikh, Thibodeaux, & Condon, 2007).

3. Results and discussion

3.1. Yield values obtained

The procedure traditionally used for the extraction of nanocrystalline cellulose is shown in Fig. 1. It involves the hydrolysis of the cellulose starting material with 64% sulfuric acid. This is done at a raised temperature of about 45 °C for a limited time, typically up to 30 min. Next, the reaction is stopped with dilution using chilled DI water. Finally, centrifugation is done and the solid product obtained is the nanocrystalline cellulose while the amorphous hydrolyzed acid-soluble products (i.e. the spent liquor) obtained are largely converted into sugars (degraded products) and disposed of.

This paper addresses a new procedure that is used for NCC extraction, also shown in Fig. 1. In this process, the principal differences are that instead of stopping the reaction with chilled water, it is slowed down by dilution with sulfuric acid at a concentration less than 64%, and the centrifugation is carried out as a two-step process. This modified process has the advantage of enabling the utilization of the spent liquor. Material recovered from the spent liquor can be used for biofuel production through hydrolysis to glucose (Martín, Galbe, Nilvebrant, & Jönsson, 2002)

As part of the typical method of obtaining NCC, when the hydrolysis reaction is stopped by quenching and dilution with cold water, this could result in the regeneration of the hydrolyzed part of cellulose together with NCC. As a result, the extraction process has been modified to avoid this co-regeneration. As per the improved method, as a consequence of diluting the hydrolyzed solution with sulfuric acid of a concentration less than 64% (i.e. 50%) and following that with centrifugation, the rate of hydrolysis is slowed down without causing cellulose regeneration in a solid form. Quenching with water or ethanol directly would precipitate cellulose II (one of the crystalline structures of cellulose) together with NCC. With the new process, however, soluble cellulose is separated from NCC and then precipitated. Moreover, to avoid the complete hydrolysis of the cellulose, the modified procedure is performed at a lower temperature but for an increased amount of time, or at the 45 °C temperature but for less time. After dilution with 50% sulfuric acid, centrifugation is used to separate the solution into two layers; one soluble in acid (the spent liquor) and the other insoluble (NCC). This selective solubility is due to sulfation (Notley, Eriksson, Wågberg, Beck, & Gray, 2006) and other factors such as degree of polymerization (Fleming, Gray, & Matthews, 2001).

Both layers are quenched with an anti-solvent: the NCC with chilled water and the spent liquor layer with chilled ethanol, to completely precipitate the cellulose and diminish any remained effect of the sulfuric acid; thus completely stopping the hydrolysis reaction. In the case of the acid hydrolyzed amorphous region (the spent liquor), it is regenerated into solid particles. The NCC and regenerated cellulose layers are then centrifuged and dialyzed, in order to neutralize their pH. The regenerated solid particles are also referred to as Biofuel Cellulose for the purpose of this paper, as an indication of their intended area of application.

Experimental runs were carried out as per the above procedure. They helped to understand the effect of the preparation parameters on the yield of the NCC and the regenerated cellulose, as well as on the Crystallinity Index of the NCC produced.

Table 1 presents the yield values for the different hydrolysis runs performed. Generally speaking, there seems to be an inverse relationship between the yield of the recovered cellulose and the temperature, so that, regardless of the concentration of sulfuric acid used in this case, the trials performed at temperatures closer to room temperature tended to give higher values for the yield of regenerated cellulose than those trials done at 45 °C. However, when comparing the NCC yields at these two categories of temperatures such a trend was not observed and the yield values remained more or less the same. Overall, it can be seen that to increase the regenerated cellulose yield it is more beneficial to perform the hydrolysis trials at room temperature. This is because at such temperatures, as a result of the kinetics of the reaction being slower, there is a more complete interaction between the acid and the cellulose during the hydrolysis and thus a more efficient breaking down of the amorphous regions of the cellulose, leading to a more efficient separation of the acid-soluble and acid-insoluble regions.

Moreover, using the ethanol as the precipitating agent for the regenerated cellulose layer helped to increase its yield, as proved in Hashaikeh and Abushammala (2011). Also, keeping the layers to settle for approximately 24 h before doing the next set of centrifugation, as was done in the hydrolysis trials performed for this paper, assisted in increasing the yield as it gives more time for the cellulose to coagulate and precipitate with the help of the precipitating agent.

In addition, for the trials carried out at 25 °C, the yield for the regenerated cellulose layer tended to increase or stay about the same as the hydrolysis time increased. However, this was not the case for the trials carried out at 45 °C, and for these experiments, the yield for the two layers actually tended to decrease as the hydrolysis time increased. This is explained by the fact that, at higher temperatures, when the hydrolysis time is increased, it eventually reaches a point at which it leads to the breaking down of the complete cellulose, leading to a reduction in the yield of both layers. This is not the case at lower temperatures where an increase in the hydrolysis time is a favorable strategy, possibly leading to a greater

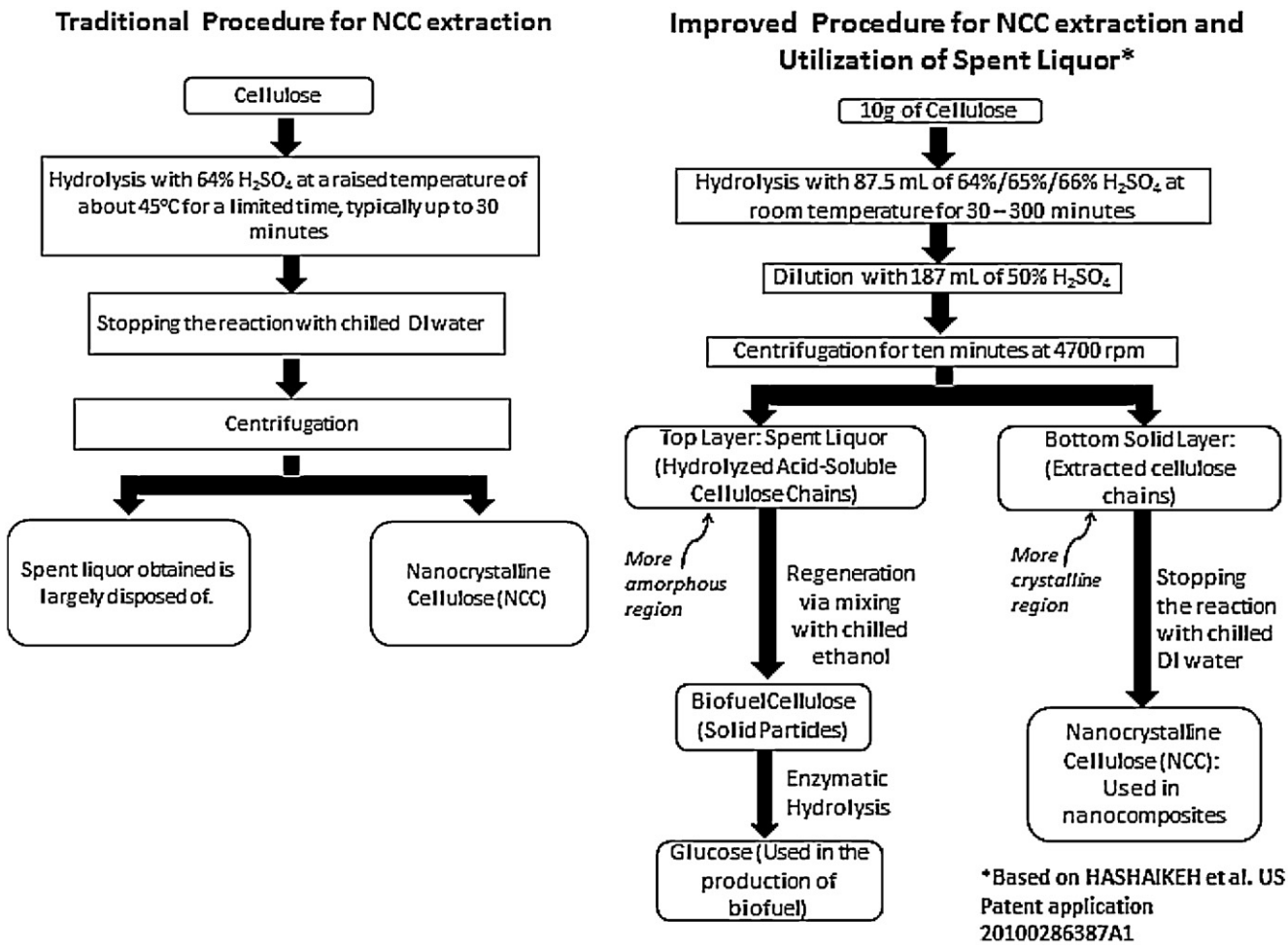

Fig. 1. The traditional and improved procedures for NCC extraction.

hydrolyzing of the amorphous cellulose regions and consequently a greater recovered cellulose yield.

Yet another parameter that was varied was the concentration of the sulfuric acid used for the hydrolysis. The different trials employed acid with concentration of 64–66%. It can be seen from the data obtained that as the acid concentration increased, the yield of the NCC layer appeared to decrease and that of the regenerated cellulose layer appeared to increase. This is the result of the fact that at stronger acidic concentrations, the amorphous regions of the cellulose are more easily broken down and this gives a greater amount of spent liquor. Since more cellulose makes up the top layer, the recovered cellulose yield is also greater. This causes less cellulose to be left in the NCC layer, and so the bottom layer yield decreases. These acid concentrations were chosen as it is known that the threshold concentration of sulfuric acid, after which even the crystalline portions of cellulose are broken down during hydrolysis, is approximately 65%. Therefore the concentrations of sulfuric acid experimented with were gradually increased with the objective of achieving a greater yield for the recovered cellulose layer while also keeping the crystalline portions of the original cellulose intact so that NCC may be obtained. These findings were supported by the XRD results discussed later on in this paper.

As a result of the above analysis, the hydrolysis run performed with 65% sulfuric acid followed by 50% sulfuric acid at 25 °C for 120 min has been selected as the optimum hydrolysis run. This is because this particular trial gave one of the greatest yields for the recovered cellulose layer as well as one of the greatest Crystallinity Index values at the most modest conditions for

Table 1
The yield values for various hydrolysis runs performed. Various conditions were used in order to optimize the yield values.

Trial #	Experimental Conditions			Yield			Crystallinity of NCC (%)
	H_2SO_4 (%)	Temp. (°C)	Time (min)	Recovered cellulose (%)	NCC (%)	Total (%)	
1	64–50	45	10	5.49	70.60	76.09	91.6
2	64–50	45	30	1.31	54.14	55.45	88.0
3	64–50	23	32	7.82	80.01	87.83	90.8
4	64–50	23	120	11.41	74.59	86.00	90.7
5	64–50	23	300	10.16	72.81	82.97	90.7
6	65–50	25	120	21.41	62.32	83.73	91.3
7	66–50	25	120	20.89	63.22	84.11	89.2
8	66–50	25	180	21.74	61.03	82.77	91.0

Crystallinity Index of starting material: 66.9%.

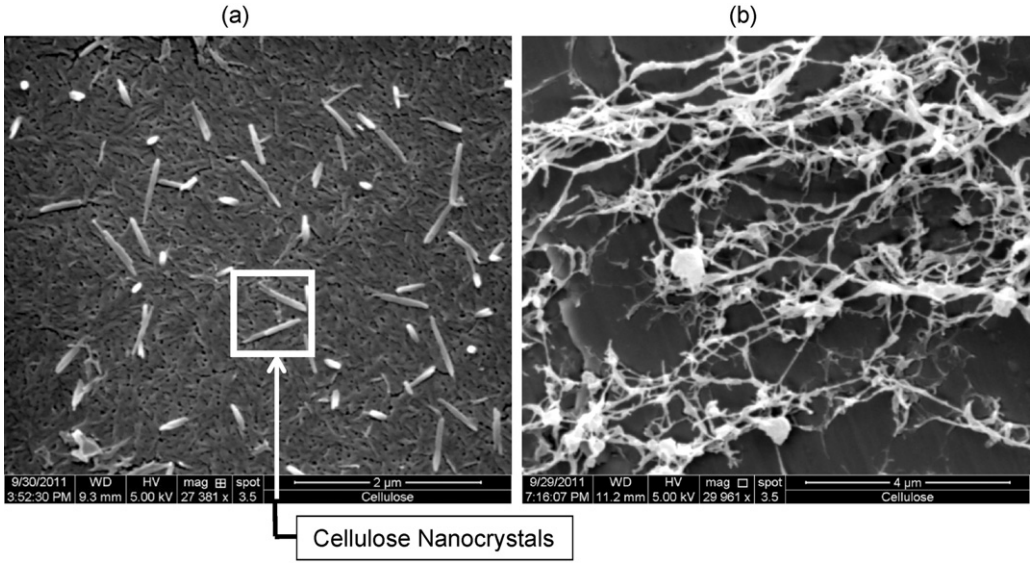

Fig. 2. (a) An SEM image of the typical NCC obtained as a result of the modified cellulose hydrolysis procedure and (b) an SEM image of the regenerated cellulose obtained as a result of quenching the spent liquor.

these particular results (i.e. a shorter hydrolysis time, relatively speaking).

Fig. 2(a) shows the SEM image taken of the typical NCC obtained as a consequence of the acid hydrolysis performed as per the modified procedure. The nanocrystals can be clearly seen in the form of a nanofibrillated top layer. They have a rod-like structure and have widths of approximately 40 nm and lengths of approximately 670 nm, yielding aspect ratios of approximately 17, and the NCC of cotton and tunicate is known to have aspect ratios in the range of 10–65 (Garcia de Rodriguez, Thielemans, & Dufresne, 2006). The nanoscale dimensions of the NCC are thus confirmed. On the other hand, Fig. 2(b) shows the open amorphous structure of the recovered cellulose.

3.2. X-ray diffraction study and the Crystallinity Index of the NCC obtained

An X-ray diffraction pattern is used in phase/substance identification and characterization since "...every crystalline substance gives a pattern; the same substance always gives the same pattern; and in a mixture of substances each produces its pattern independently of the others" (Hull, 1919). Broad humps represent amorphous portions of the material under test and sharp peaks represent crystalline regions. The Crystallinity Index can thus be calculated using the Segal Method, as already described in Section 2.4 of this paper. The Crystallinity Index values thus calculated for the NCC obtained during the different hydrolysis trials performed are also mentioned in Table 1. The Crystallinity Index of the starting material must also be noted, in order to analyze to what extent the hydrolysis run led to an increase in the Crystallinity Index of the NCC layer. For the cotton cellulose (filter paper) starting material, the Crystallinity Index was 66.9%. At the same time, for all the hydrolysis trials performed, the Crystallinity Index of the NCC layer was at least 88%. This is an increase in the crystallinity of the cellulose by more than 22%. This proves the statement that the acid hydrolysis performed separated the cellulose into its more crystalline (NCC) and more amorphous regions.

When studying those trials performed at room temperature, the observation is made that regardless of the hydrolysis time or the acid concentration in this case, the Crystallinity Index of the NCC layer turned out to be more or less the same. 23 °C/25 °C are less severe temperatures; the crystallinity of the final product remains intact even when the hydrolysis time is increased. In fact, this is not the case with the hydrolysis trials performed at 45 °C, where, relatively speaking, the difference in the Crystallinity Index of the NCC obtained by the two trials is more substantial. Thus the longer hydrolysis trial seemed to have a detrimental effect on the NCC layer, breaking down more of it and making it less crystalline.

In addition, it is found that there is no significant correlation between the yield values of the NCC layers and their Crystallinity Index values. The Crystallinity Index obtained for the NCC layers is heavily dependent on the Crystallinity Index of its starting material and not as dependent on the hydrolysis parameters. The only possible exception for this was the hydrolysis run performed for half an hour at 45 °C, as already explained.

Fig. 3 shows the XRD pattern of the cotton cellulose starting material as well as the XRD pattern for the NCC obtained by the experiment performed at 45 °C for 10 min. Since the Crystallinity

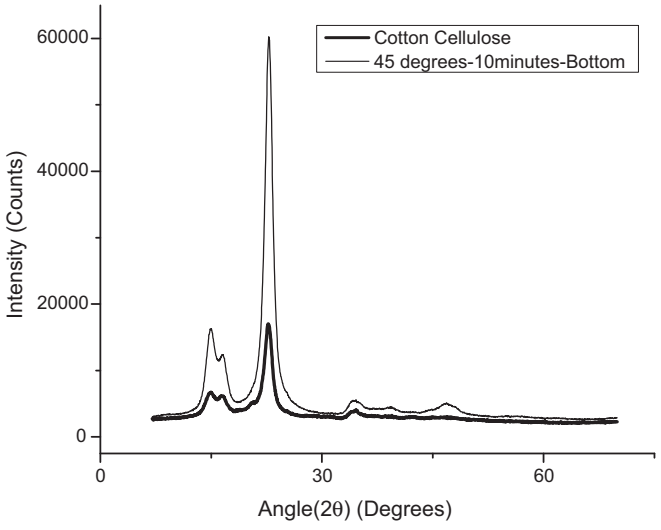

Fig. 3. The XRD patterns for the cotton cellulose material used in the experiments performed, and for the NCC obtained by the trial carried out at 45 °C for 10 min.

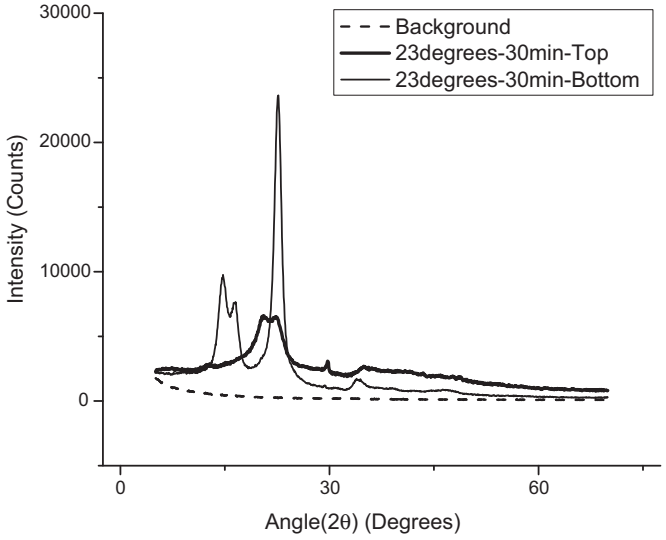

Fig. 4. The XRD pattern for the recovered cellulose and NCC layers of the hydrolysis trial performed at 23 °C for half an hour.

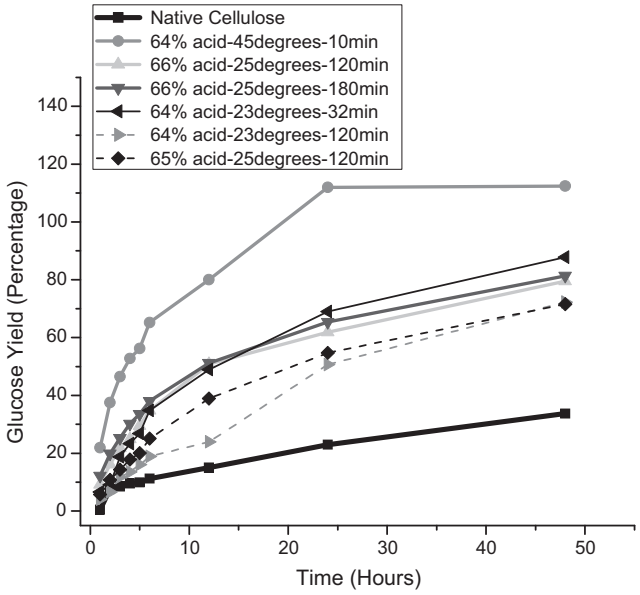

Fig. 5. Enzymatic hydrolysis profile of the recovered cellulose layer for various trials.

Index is broadly represented by the steepness of the peak, it is clear how the NCC obtained is significantly more crystalline than the stating material of cotton cellulose. This is expected since the purpose of the hydrolysis is to separate the cellulose into its more amorphous and more crystalline regions, thus obtaining NCC.

When native cellulose (Cellulose I) is dissolved, it regenerates into Cellulose II, a more thermodynamically stable state (Hermans & Weidinger, 1946; Xiang et al., 2003). This observation can be verified by XRD data. The characteristic XRD curves of Cellulose I and Cellulose II are well known. As a result, when considering the XRD patterns for the recovered cellulose and NCC layers of the hydrolysis trial performed at 23 °C for half an hour (Fig. 4), it can be seen how the regenerated cellulose layer is Cellulose II (the thick solid line) while the NCC layer is Cellulose I (the thin solid line). The dashed curve represents the background scan and thus must be subtracted from the NCC and regenerated cellulose curves to get the actual intensity values for the materials being studied. What is more, it must be stated that the Crystallinity Index of the regenerated cellulose has not been calculated since its XRD scan is characteristic of Cellulose II and reflects the amorphous structure of the material.

3.3. Enzymatic hydrolysis of the recovered cellulose layer

The enzymatic hydrolysis of the Cellulose II (Biofuel Cellulose) layers was performed for the various trials performed as part of this work. These layers were tested as it is the regenerated cellulose chains found in them which are intended to be used in biofuel production. As can be seen from the curves of Fig. 5, this yielded positive results with a complete conversion of glucose being achieved in about 17 h for the trial performed at 45 °C and approximately 80% conversion being achieved in 48 h for the trials performed at room temperature conditions. This is much faster than performing the enzymatic hydrolysis on native cellulose, 50% of which did not convert into glucose even 40 h after the reaction began. Thus, the recovered cellulose yielded rates of conversion more than double those of native cellulose. The greater susceptibility of the regenerated cellulose to the enzymatic hydrolysis is due to the fact that it is Cellulose II (unlike the native cellulose which is Cellulose I). This entails that it has a low degree of polymerization and a high surface area as well as a more open structure, as shown in the SEM image of Fig. 2(b). These features help to increase the rate of enzymatic hydrolysis and help to decrease the cellulase enzyme requirement, making the production of glucose from cellulose in this way faster and less expensive and therefore more feasible. In addition, the faster rate of enzymatic hydrolysis of the trial at 45 °C relative to those trials performed at room temperature is expected since the increased temperature would cause the amorphous cellulose to be more completely hydrolyzed and thus have an even more open structure. The above data thus proves the practicality of using the amorphous cellulose obtained from the spent liquor of acid hydrolysis in the production of biofuel.

4. Conclusions

This paper presents a novel process which can be employed to recover the high value material from the spent liquors produced as a byproduct during the sulfuric acid hydrolysis of cellulose. It explains the extraction of nanocrystalline cellulose (NCC) using acid hydrolysis with sulfuric acid with concentration of 64–66%. After the hydrolysis, the suspension produced is separated into two constituents with the help of centrifugation: the more crystalline portion is NCC, and the less crystalline portion leads to the production of regenerated cellulose. The various parameters which influence the yield of each of these constituents are studied. It is therefore concluded that a higher yield for the regenerated cellulose layer can be obtained at room temperature hydrolysis conditions. These positive results are not obtained when performing the hydrolysis runs at a heightened temperature of 45 °C. The hydrolysis run performed with 65% sulfuric acid followed by 50% sulfuric acid at 25 °C for 120 min has been selected as the optimum hydrolysis run.

X-ray diffraction (XRD) data of the materials formed is analyzed, indicating that the cellulose of the NCC layers is essentially extracted from the raw materials and is not regenerated, thus having the characteristic Cellulose I structure. On the other hand, the cellulose of the spent liquor is regenerated and thus gives XRD patterns characteristic of Cellulose II. The Crystallinity Index of the NCC is also significantly greater than the values of the same parameter for its starting material.

Enzymatic hydrolysis of the regenerated cellulose layer is carried out. The rate of this reaction is found to be fast. This indicates the practicality of producing glucose in this way. In addition, with

the relatively high yield values of the regenerated cellulose layer obtained, and with the possibility of further yield increases as per the optimum hydrolysis conditions being investigated, the suitability of using the regenerated cellulose layer in biofuel formation is highlighted. Moreover, using the NCC as a component in composite materials is also delineated due to its excellent mechanical properties. This introduces a combined technology concept which focuses on generating a high value product (NCC), the cost of which pays for a low value product (e.g. biofuels). The low value product is rich to the environment.

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