Chemically purified cellulose and its nanocrystals from sugarcane bagasse: isolation and characterization

Suter K. Evans, Omwoyo N. Wesley, Oyaro Nathan, Makwena J. Moloto

Abstract

Agro-wastes such as sugar cane bagasse can be explored for use in different aspects. Its applicability as a source of cellulose has attracted much interests especially in biomedical field among various applications. In the current work chemically purified cellulose (CPC) and cellulose nanocrystals (CNCs) were effectively extracted from sugarcane bagasse (SCB). The cellulose was obtained by chemical treatment of SCB using HNO3, NaOH and a bleaching agent. Nanocrystals were further prepared from the extracted cellulose using H2SO4 hydrolysis followed by washing with deionized water and acetone. The obtained materials were characterized for surface morphological using Fourier transform infrared (FTIR) spectroscopy, Transmission Electron Microscopy (TEM) and X-ray diffraction (XRD) analysis. The thermal properties were evaluated using TGA/DTG. The FTIR showed the disappearance of the peaks responsible for the hemicelluloses and lignin. These results were confirmed by TGA which proved gradual elimination of non-cellulosic constituents. X-ray Diffractometer depicted an increase in crystallinity occasioned by sequential treatments to get the cellulose nanocrystals. Cellulose nanocrystals had a spherical shape with a diameter of 38nm as compared to the chemically purified cellulose which had a diameter of 76nm. The CNCs prepared with this method were seen to be less agglomerated and more crystalline thus possess a higher potential as bionanocomposite either for biomedical applications or for wastewater treatment among other industrial application. This approach also provides an opportunity for the sugar companies to effectively manage their waste product.

1. Introduction

Cellulosic nanomaterials have gained increasing attention as agents for biocomposites for industrial and biomedical applications due to their sustainability and renewability (Faruk et al., 2012). Their surfaces are rich with (-OH) groups that can be chemically modified (Plackett, 2010). They are characterized with low density, high aspect ratio, good mechanical features and faint toxicity (Lavoine et al., 2012). Several studies on nanomaterials has been extensively done for an extensive potential use; scaffolds for tissue engineering, filters, membranes for water treatment, transparent films, adhesives, drug delivery and antimicrobial films, among other uses (Moon et al., 2011). Cellulose is a natural water permeable agro-based polymer comprised hydroxyl functional groups that enhance the hydrogen bonding. Cellulose can be obtained from cotton, sisal, Phormium tenax leaf fibers, corncob, banana rachis, rice husk, soy hulls, cassava bagasse and sugarcane bagasse (Kumar et al., 2014). Research studies on the use of cellulosic waste for various functions have grown rapidly. Crystalline cellulose has been previously been extracted from cornstalks (Reddy and Yang, 2005), cassava bagasse (Pasquini et al., 2010), coconut husk (Rosa et al., 2010), banana rachis (Zuluaga et al., 2009), soybean pods (Wang and Sain, 2007), mulberry bark (Li et al., 2009), wheat and soy hulls (Alemdar and Sain, 2008, Johar and Dufresne, 2012). Other major components of plants include hemicelluloses and lignin. Lignin is more of a cementing-matrix for cellulose and hemicellulose with Ester-type bond linkage that is both very sensitive and insensitive to alkali treatments. The cellulosic materials are known to be crystalline while lignin and hemicellulose are reported to be amorphous (Kumar et al., 2014). Cellulosic materials can be classified into cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs) which can also be referred as nanofibrillated cellulose (NFC) or microfibrillated
Cellulosic nanomaterials have been used to prepare composites with higher mechanical strength (Lu and Hsieh, 2010). Cellulose nanofibrils have been used as carriers for delivery of drugs (Johar et al., 2012) among many other applications. The major challenge of preparing the cellulose nanocrystals for further applications is their tendency to aggregate. The hydrogen bonds of cellulose pull the cellulose nanocrystals together and make their re-dispersion difficult thus hindering their effective processing (Kumar et al., 2014). Various methods have been used to prepare cellulose nanocrystals which include ultrasonic technique, acid hydrolysis, and enzymatic hydrolysis (Mashego, 2016) though the acid hydrolysis has been mostly used since the method is simple and fast to generate cellulose nanocrystals. In other studies cellulose nanofibrils were prepared from bagasse pulp through ultra-micron grinding and high pressure homogenization using xylanase enzyme and cold alkali for pre-treatment (Nie et al., 2018; Tao et al., 2019). It is therefore necessary to develop suitable methods to isolate the cellulose nanocrystals that will be free from aggregation and Ostwald ripening.

There are several agro-based industries that produce huge amounts of plant biomass. Sugarcane is the world's largest crop by production quantity, with approximately 1.9 billion tonnes produced yearly (Otierno, 2015). In Kenya Sugarcane is mainly grown in the former western and Nyanza provinces. The industry has eleven operational sugar factories namely, Chemell Sugar Factory; Kibos Sugar and Allied Factories; Muhoroni Sugar Factory; Mumias Sugar Factory; Nzoia Sugar Factory; Soin Sugar Factory; South Nyanza Sugar Factory; Sukari Industries Limited; Transmara Sugar Factory; West Kenya Sugar Factory and Butali Sugar Factory (Otierno, 2015). Sugarcane baggase is an agro-based dry pulpy residue after the extraction of juice from cane from these industries. Bagasse has been reported to cause tremendous environmental pollution in the areas located with sugar industries since they are scattered all over making the scenery of these areas to look ugly. The bagasse wastes produce bad odors when it ferments making the environment not conducive to reside on. Furthermore, these wastes cost the industries on storage space and disposal costs. The sugar industries generate huge quantity of bagasse during the manufacture of sugar from cane. This phenomenon results to environmental distress, also triggering numerous environmental extortions instigating damage to the land and its surroundings (Masayi and Netondo, 2012). Diverse methods for bagasse disposal, including using it for power co-generation and manufacture of molasses which is used for industrial production of ethanol have been explored by the factories. The sugarcane bagasse has also been explored for the manufacture of briquettes though all this use has not addressed the menace of bagasse (Otierno, 2015). Other uses of sugarcane bagasse comprise exploitation of its cellulose content (Jacobsen and Wyman, 2000) for further application either in water treatment or other relevant uses to the very industries or for sale. Thus, the extraction of cellulose from sugarcane bagasse for the production of cellulose nanocrystals was sought.

Therefore this study explored the extraction of cellulose from sugarcane bagasse and to isolate cellulose nanocrystals from the prepared cellulose. The raw bagasse, chemically purified cellulose and the cellulose nanocrystals were characterized for surface morphology using TEM, and FTIR, crystallinity analysis using XRD and thermal properties using TGA.

1.1. Statement of hypothesis

Extraction of cellulose from sugarcane bagasse can be enhanced by the use of nitric acid as a starting material for digestion. Cellulose nanocrystals can further be prepared from extracted cellulose for enhanced properties and applications.

2. Materials and methods

2.1. Materials

The Sugarcane bagasse which is an agricultural waste was collected from South Nyanza Sugar Company, Awendo, Kenya (Sony Sugar Company). Nitric acid HNO₃ (≥98.99%), Sodium hypochlorite (NaClO), Sulfuric acid H₂SO₄ (≥99.9%), acetic acid, sodium hydroxide and acetone (≥99.9%) were used and were of analytical grade. Deionized water was used all through the entire experiment.

2.2. Methods

2.2.1. Isolation of chemically purified cellulose

Sugarcane bagasse was primarily washed with deionized water to remove any unwanted particles. It was then sun-dried before grinding into small pieces using a blender and sieved under double mesh sieves. The powdered bagasse was oven dried at 105 °C for about 5 h and then stored at room temperature in air tight polythene bags. The isolation of the cellulose was done according to previously documented methods (Johar et al., 2012) but with some modifications. Briefly, 70 g of the bagasse powder was mixed with 700 mL 6% (w/v) of HNO₃ for 2 h in a hot water bath placed at 80 °C then washed with deionized water until neutral. The solution was then refluxed with 500 mL of 1% NaOH at constant stirring for 2 h at 80 °C in a hot water bath. It was further washed with deionized water and bleached with 250mL of sodium hypochlorite 0.735% (w/v). Acetic acid was further added to the ligno-cellulosic extract and stirred for 2 h at 80 °C. The residue was then washed with deionized water until a neutral pH and left to dry for three days at room temperature.

2.2.2. Preparation of cellulose nanocrystals (CNCs)

The isolated cellulose was used in literature (Kumar et al., 2014) though with slight modifications also. The chemically CPC from SCB was hydrolyzed with 32% (w/v) of H₂SO₄ with a 1:25 g/mL ratio of cellulose to the dilute acid at room temperature for 24 h under constant stirring. This reaction was then quenched by addition of 10 fold deionized water to the reaction mixture, followed by centrifugation at 10,000 rpm for 15 min three times to remove the acidic solution. The supernatant was discarded and the cellulose precipitate re-dispersed in deionized water and dialyzed against deionized water several times. The colloidal suspension was then sonicated in an ice bath sonicator for 1 h to homogenize the generated cellulose nanocrystals. The generated nanocrystals were further centrifuged at 6, 000 rpm for 30 min. This was then allowed to settle for 24 h then the water was replaced with acetone and centrifuged at 6,000 rpm for 30 min. The cellulose nanocrystals were finally oven dried in vacuum at 70 °C overnight.

2.3. Characterization of SCB, CPC and CNCs

2.3.1. Fourier transform infrared spectroscopy (FTIR)

This technique was used to manipulate structural changes on samples as a result of chemical modification by the identification of the functional groups. The changes in functional groups of the materials; SCB, CPC and CNCs were investigated using FTIR spectroscopy using Nicolet, i50, FT-IR (Thermo Nicolet, USA) spectrophotometer. The FTIR spectra of the samples were recorded in the transmittance mode in the range of 4000 cm⁻¹ to 500 cm⁻¹.

2.3.2. Transmission Electron Microscopy (TEM)

Morphological properties and particle sizes of CPC and CNCs were determined using (Tecnai G2 20 S-twin) Transmission Electron Microscope. The samples were dispersed in a suitable medium and then placed on a copper grid coated with a carbon film. Thereafter, the samples were then dried before carrying out TEM analysis at an accelerating voltage of
100–120 kV.

2.3.3. X-ray diffraction (XRD)

The crystallinity index of the SCB before and after chemical modification was analyzed using Shimadzu XRD-700 X-RAY Diffractometer. SCB, CPC and CNCs in form of milled powder were placed on steel sample holders and leveled to obtain total and uniform X-ray exposure. The samples were analyzed at 25 °C with a monochromatic CuKα radiation source χ = 0.154 nm with a 2 theta angle ranging from 10° to 60°. The crystallinity index was calculated using the equation below (Johar et al., 2012).

\[ C, I(\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \]

Where, \( I_{002} \) denotes the maximum intensity of the 002 lattice diffraction peak and \( I_{am} \) is the minimum intensity scattered by the amorphous part of the sample (Johar et al., 2012).

2.3.4. Thermogravimetric analysis (TGA)

To confirm the removal of hemicellulose and lignin, TGA measurements were performed for SCB, CPC and CNCs using a Mettler Toledo thermogravimetric analyzer (TGA/SDTA 85-F). Under all measurements a mass of 12 mg was used in each analysis. In addition, the thermal measurements were carried out with a gas flow rate of 10 mL min⁻¹ heating from 30 °C to 900 °C under nitrogen atmosphere.

3. Results and discussions

3.1. Physical appearances

The physical appearances of the raw sugarcane bagasse that was collected from the factory, the powdered bagasse, the chemically purified cellulose and its nanocrystals prepared by hydrolysis method and its crystallinity index of the SCB before chemical modification was analyzed using Shimadzu XRD-700 X-RAY Diffractometer. SCB, CPC and CNCs in form of milled powder were placed on steel sample holders and leveled to obtain total and uniform X-ray exposure. The samples were analyzed at 25 °C with a monochromatic CuKα radiation source χ = 0.154 nm with a 2 theta angle ranging from 10° to 60°. The crystallinity index was calculated using the equation below (Johar et al., 2012).

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The structural change of the SCB was determined by FTIR before and after chemical treatment. The Infra-Red spectra of SCB, CPC and the CNCs are captured below in Fig. 2. The broad peak at 3100-3500 cm⁻¹ indicates the O–H stretching bonds while the peak around 2800-2950 cm⁻¹ indicates the C–H stretching. The peak around 1750 cm⁻¹ corresponds to C=O bond which is normally found in the linkages of the esters in the hemicellulose and lignin. The peaks observed around 1600-1700 cm⁻¹ indicates the aromatic ring found in the lignin. The peak between 1200-1300 cm⁻¹ depicts an out of plane C–O stretching in the aryl group of the lignin linked to the SCB before chemical modification. The modification of SCB with sodium chloride and sodium hypochlorite lead to the disappearance the bands, FTIR spectrum of CPC and CNCs (Fig. 2). The FTIR spectrum of CNCs is similar to that of CPC but it has sharp bands. The peaks observed at 1600–1650 cm⁻¹ for the CPC and CNCs are as a result of O–H bending due to adsorbed water (Johar et al., 2012), the bands between 1400-1450 cm⁻¹ is attributed to CH₂ inter-twine in the cellulose material. The peak at 1050 cm⁻¹ indicates the C–O–C pyranose ring stretching vibration (R. Liu, Yu and Huang, 2005). The peak observed around 900 cm⁻¹ is reported to be associated with the cellulose β-glycosidic linkages (Reddy and Yang, 2005). These results depicted that the cellulose molecular structure remains unaffected following chemical treatment of SCB.

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3.3. X-ray diffraction (XRD) analysis

The X-ray diffraction patterns of the SCB, CPC and CNCs are shown below in Fig. 3. The crystallinity index of SCB, CPC and CNCs was calculated from the XRD. Crystallinity index (CRI) denotes the ratio of the crystalline constituents to the amorphous regions of a material (Kumar et al., 2014). The SCB, CPC and CNCs exhibited three main characteristic peaks around a 2θ value of 16.1°, 22.1° and 37.5°. The peaks represent the characteristic patterns for the crystal form of cellulose I polymorph since there is no doublet peak around 2θ value of 22° (Klemm et al., 2005). The peak at 2θ value 16.1° corresponds to (110) crystallographic plane while the one at 2θ value of 22.1° and 37.5° relate to the (002) and (004) crystallographic planes correspondingly (Johar et al., 2012). After the treatment, more intense crystalline peaks were observed with the main reflections at 2θ value of 22.1° and 37.5°. This was also a confirmation of the successful removal of the lignin from the bagasse. The samples presented high peak intensity around 2θ value of 22.1° which is correlated to the crystalline structure of cellulose. Also, from the XRD patterns, presence of a broad peak at 2θ around 16° is characteristic to the amorphous arrangement.

![Fig. 1. Physical appearance of collected sugarcane bagasse (a), crushed sugarcane baggase (b), extracted cellulose (c) and nanocrystals (d).](image-url)
The crystallinity index (CrI) of SCB to CPC to CNCs that were calculated for the XRD patterns presented an increase in crystallinity SCB > CPC > CNCs (Table 1). From the experimental data SCB presented the lowest CrI due to higher amounts of the amorphous constituents. With chemical modification using sodium hydroxide and sodium hypochlorite in extraction of the cellulose, crystallinity increased. This is attributed to successful elimination of hemicelluloses and lignin that were attached to the cellulosic bagasse. Crystallinity index was observed to increase from SCB to CPC after acid hydrolysis using sulfuric acid. The increase in crystallinity upon acid hydrolysis depicted the dissolution of the amorphous region of the SCB. During the chemical treatment, sulfuric acid reacts with the amorphous region of SCB causing hydrolytic cleavage of the glycosidic bonds thus releasing individual crystallites. This in turn causes the growth of monocrystals which contributes to the increase in crystallinity which is observed as narrow and pronounced diffraction peaks in the XRD curve. Similar results were obtained by Johar et al. (2012), where they extracted cellulose nanocrystals from rice husks. Their untreated rice husks and the treated ones showed characteristic peaks typical of cellulose I with three well defined crystalline peaks around 2θ value of 16°, 22° and 35°. The peaks equally became more defined upon each step of chemical treatment. In other studies, similar patterns were observed when the cellulose nanocrystals was extracted by chemical treatments of other agricultural wastes including from wheat straw (Liu et al., 2005), from oil palm mesocarp fiber (Chieng et al., 2017), from corn Stover (Costa et al., 2015) and even from Agave

### Table 1
Crystallinity index of SCB, CPC and CNCs.

| Sample | Crystallinity Index (CrI%) |
|--------|---------------------------|
|        | 2θ (Amorphous) | 2θ (002) |
|        | Degree | Intensity, Iam | Degree | Intensity, I002 |
| SCB    | 16.34 | 394          | 22.38 | 664          | 40.66 |
| CPC    | 16.12 | 292          | 22.38 | 892          | 67.26 |
| CNCs   | 16.32 | 214          | 22.34 | 926          | 76.89 |

Fig. 2. FTIR spectra for SCB, CPC and CNCs.

Fig. 3. X-ray diffraction patterns of SCB, CPC and CNCs.
The thermal property of any given sample is an essential feature in the determination of the response to a mass change with respect to temperature. Thermogravimetric (TG) curves displayed weight variations under heating with respect to the derivative curve displaying differences in the TG slope that cannot be easily noted with TGA curve. From the DTG curves differences in temperature can indicate on whether the thermal process is exothermic or endothermic. The TGA and DTG thermal properties of the bagasse, extracted cellulose and the nanocrystals are shown in Fig. 4 below. This was performed to investigate the suitability of SCB and the cellulose compounds. The thermal behavior of the lignocellulosic materials depends on their chemical composition, structure and degree of crystallinity (Rosli et al., 2013). The thermal decomposition parameters were determined from TGA as shown in the graphs. The TGA curve for the SCB showed four degradation steps which are characteristic of moisture content, hemicellulose, cellulose and lignin degradation. The initial weight loss started at about 40 °C for the three samples and this went on until a maximum temperature (T_{max}) of 128 °C. This degradation was attributed to the loss of moisture content of the samples. The graphs depict that CPC had the highest moisture content of around 8% while the SCB and CNCs have a moisture content of about 3% each. The second degradation step for SCB starts around 200 °C which is a starting temperature for the lignin degradation.

The lignin decomposes very slowly over a broader range of temperature (200–800 °C) than the cellulose and hemicellulose components of the biomass (Brebu and Vasile, 2010). Previous studies have documented DTG curves of lignin decomposition as having flat peaks with a sloping baseline which makes it impossible to define activation energy for the reaction since there is a flat tailing section at higher temperatures (H. Liu, Liu et al., 2010). This phenomenon is different for the sharper and pronounced DTG peaks of cellulose and hemicellulose. Brebu and Vasile (2010) explained the non existence of the degradation peak responsible for lignin. Since the heating rate is 10 °C/min and the lignin decomposes very slowly at approximately less than 0.15 wt%/°C, the mass lost is only 40 wt% of its initial mass below 700 °C. The degradation rate then slightly increases to 0.3 wt%/°C above 750 °C and finally the mass loss at around 850 °C is documented to be approximately 67 wt% (Yang et al., 2006). The degradation for SCB recorded between (204–300 °C) with a T_{max} around 280 °C is attributed to the degradation of hemicelluloses (Rosli et al., 2013). The degradation peak observed at a T_{max} around 350 °C in the SCB curve is attributed to the cellulose degradation. The degradation of the CPC occurred in the range of 224–357 °C with a T_{max} observed at about 320 °C while the CNCs degraded in the range of 240–341 °C with a T_{max} around 310 °C.

Generally, the degradation temperature is seen to decrease when the SCB is chemically treated and hydrolyzed. This is attributed to the removal of hemicelluloses and lignin as well as the crystallinity of the CPC and CNCs which is higher than SCB. Basically, increase in crystallinity is documented to improve heat resistance and this leads to an improvement in the thermal stability of a material (Rosli et al., 2013). The further reduction of the degradation temperature of the CNCs may also be attributed to the introduction of the sulfate groups into the cellulose crystals during the sulfuric acid hydrolysis process (Lavoine et al., 2012). The residue left after complete degradation of the samples were observed to be about 19.6% for the SCB while CPC and CNCs had only 14.8% left for each. The higher residual amounts recorded for SCB is attributed to the presence of lignin which as earlier said, have a very slow degradation rate and might not be completely burnt (Johar et al., 2012). The amounts of residue recorded for CPC and CNCs are slightly lower signifying the successful removal of the lignin (Rosli et al., 2013).

3.5. Transmission Electron Microscopy

Transmission Electron microscopy was used to study the morphology and determine the particle sizes of the chemically purified cellulose and cellulose nanocrystals. The chemical treatment of the SCB with an alkali solution and further by acid hydrolysis was expected to remove the hemicelluloses and lignin which form part of the amorphous region of the cellulose bagasse (Ahmed et al., 2005). This process is aimed at reducing the size of the cellulosic nanomaterials to nanometer range while leaving the crystalline regions intact. Micrographs from the TEM analysis of CPC and their mean particle size is shown in Fig. 5 while that of CPC is shown in Fig. 6.

The CPC had an average size of 76 nm in comparison to that of CNCs with 38nm average particle size. From the TEM image of CPC, it can be observed that the particles are having short ‘rod like’ shapes which have

![Fig. 4. TGA(a) and TDG (b) curves of SCB, CPC and CNCs.](image-url)
undergone Ostwald ripening. The CNCs on the other hand have a spherical shape with little agglomeration observed as compared to other studies. This agglomeration is attributed to the surface ionic charge that made the crystallites get stacked together due to acid hydrolysis process (Liu et al., 2010). The observed agglomeration may also be due to TEM sample preparation process once the dispersing medium was removed (Pires et al., 2013) though this agglomeration was seen to reduce significantly for the CNCs. The cellulosic chains are known to have hydrogen bonds and also some hydrophilic interaction between the chains which might also be responsible for the observed agglomeration (Kumar et al., 2014).

In other similar studies, Rosli et al. (2013), prepared CNCs from Agave angustifolia fiber that showed needle like structure consisting of some fibrils and some aggregates. These fibrils had an average diameter of 10 nm with a length of 310 nm Chieng et al. (2017), also observed agglomerated crystals of cellulose from Oil palm mesocarp which had an approximate diameter of 5 nm. Nanocellulose was also prepared in another study by acid hydrolysis of isolated cellulose from sugarcane bagasse using varied concentrations of sulfuric acid (Wulandari et al., 2016). He equally observed agglomerated spherical shaped nanocellulose having a diameter of about 197 nm. In most studies the agglomeration was attributed to the surface ionic charges which made the crystals to stick together after the hydrolysis process.

4. Conclusion

The study confirms cellulose can be obtained from sugarcane bagasse. The removal of non-cellulosic compounds can be enhanced by the use of nitric acid during chemical treatment and washing with acetone after sulfuric acid hydrolysis. An increase in crystallinity was observed for the nanocrystals which indicated the exposure of the crystalline phase after successful elimination of the lignin and hemicelluloses. The particle size greatly reduced in diameter after the acid hydrolysis of cellulose as observed by TEM and this is an indicator in improved properties of the CNCs. The results obtained herein suggest that SCB which is a menace in all the sugar factories can be utilized effectively for several other aspects since they possess the advantages of being sustainable and biodegradable.

Declarations

Author contribution statement

Wesley Nyaigoti Omwoyo: Conceived and designed the experiments;
Wrote the paper.
Evans K Suter: Performed the experiments.
Nathan Nyaigoti Oyaro: Analyzed and interpreted the data.
Makwena J Moloto: Contributed reagents, materials, analysis tools or data.

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