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A new method for producing microcrystalline cellulose from *Gluconacetobacter xylinus* and kenaf

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ABSTRACT

A new preparation method of microcrystalline cellulose from *Gluconacetobacter xylinus* (BC) and kenaf (KF) is reported. The developed cellulose (DBC and DKF) materials showed different crystalline structures. DBC exhibited cellulose I lattice with high crystallinity (85%) whereas DKF showed cellulose II lattice with high crystalinity (70%). The particle size of DKF was 5–20 μm while that of DBC was 1–5 μm. The physical properties of the DBC and DKF materials were compared with those of the commercially available microcrystalline cellulose Avicel®PH 101. DBC exhibited lower value of the loose density than those of DKF and Avicel PH 101. Both microcrystalline DBC and Avicel PH 101 demonstrated similar behavior during flow and binding processes. The thermal properties of DBC and DKF materials were investigated by thermogravimetric analysis. The TGA results reveal increased thermal stability for DBC compared to DKF. Moreover, the weight loss of DBC occurred in one step degradation process from about 320 °C to 380 °C, which is mainly due to the decomposition of cellulose.

1. Introduction

Cellulosic materials are widely used as filling and binding agents in direct compression of tablets due to their fibrous structure of their flow ability. Many commercial cellulose products with different particle and tableting properties are available. The differences in particle structures and thus in powder properties of these materials are often due to differences in manufacturing processes (Kumar, Medina, & Yang, 2002). Some of the cellulose materials are mechanically aggregated and are of excellent flow properties, they are more or less of poor binding properties (Kumar et al., 2002). However, microcrystalline cellulose produced by acidic hydrolysis and spray drying processes are widely used as tableting adjuvant and it is proved to be an excellent binding agent (Bolhuis, Veen, Wu, Zuuraman, & Frijlink, 2005). Nevertheless, flow difficulties still exist, especially when larger amounts of microcrystalline are added into the tablet masses (Paronen, 1983). A new direct compression excipient called low crystallinity cellulose ranging in degree of crystallinty from 15 to 45% has been developed; it is produced by reacting cellulose with phosphorous acid first at room temperature for 1 h and then 45–75° for 2–10 h, followed by adding the resulting highly viscous solution to water (Wei, Kumar, & Banker, 1996). The use of alkali hydroxides as swelling agents for cellulose has been extensively investigated (Krassig, 1996; Kumar et al., 2002; Lin, Conner, & Charles, 1992). Kumar et al. (2002) investigated the use of sodium hydroxide treated cellulose powder as a direct compression excipient, where it showed the cellulose II lattice of low crystallinity 47% and higher true, bulk, and tap densities than those of Avicel PH 102. Kothari (1998) reported that microcrystalline cellulose exhibited different powder and mechanical properties depending on the agitation rates. Extracellular synthesized bacterial cellulose (BC) differs from plant cellulose and exhibits good physical properties, high crystallinity, high water absorption capacity and better mechanical strength (Gouda & Keshk, 2010; Keshk, 2006). Kenaf (*Hibiscus cannabinus* L.) is an annual herbaceous crop of the Malvaceae family, of which cotton and okra are also members. Kenaf is believed to have its origin in ancient Africa (Western Sudan) and it has been cultivated in Egypt as early as 4000 B.C. (Kobayashi, 1991). The kenaf bast readily pulped and produces a high quality pulp comparable to that produced from softwood, whereas the core is richer in lignin, gives pulp with poor strength characteristics (Keshk, Suwinarti, & Sameshima, 2005, 2006). The investigation of new application for Kenaf is required to have a good use of it. The objective of the present study is to prepare and characterize microcrystalline cellulose produced from *Gluconacetobacter xylinus* and Kenaf using acidic hydrolysis followed by treatment with sodium hydroxide solution. Moreover, the physical properties of the prepared cellulose materials compared with those of Avicel PH 101 are discussed.

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Table 1
Relation between hydrochloric acid concentration and the newly developed cellulose from kenaf (DKF) and bacterial cellulose (DBC).

Volume HCl/1000 ml H_2O	%Yield of DKF	%Yield DBC
36	–	–
56	30	–
76	37	–
96	50	–
100	62	15
108	66	25
116	66	25

2. Materials and methods

2.1. Materials

A frost-killed kenaf variety Tainung-2 grown was harvested (180 days after planting). The stems were separated manually into bast and core. The bast fibers were cut into 3 cm lengths prior to treatment. American Type Culture Collection (ATCC) is the supplier of the *G. xylinus* ATCC 10245. Cellulose Avicel PH 101 was purchased from Fluka.

2.2. Methods

2.2.1. Culture medium and conditions

The Hestrin–Schramm (HS) culture medium was used (Hestrin and Schramm, 1954). It was composed of 2.0% D-glucose, 0.5% peptone, 0.5% yeast extract, 0.12% citric acid, and 0.27% disodium hydrogen phosphate. The culture was incubated statically at 28 °C in the liquid medium at pH 6 for one week.

2.2.2. Pellicles production and purification

Cellulose membrane was produced by *G. xylinus* strain in 30 ml of the HS medium at 28 °C for 7 days using 90-mm (i.d.) Petri dishes. After incubation, the pellicles produced on the surface of each medium were separated by centrifugation. For removal of microbial product contaminants, the pellicles were washed with water and with 10% sodium hydroxide for 30 min and then neutralized by 6% acetic acid and water successively. After that, the pellicles were dried in an oven at 105 °C to constant weight.

2.2.3. Pulping method

Pulping treatment of Kenaf was carried out using combination of ammonium oxalate and sodium hydroxide by refluxing followed by acidic chlorite, (Keshk et al., 2005, 2006). Kappa number and cellulose (as α-cellulose) were determined by TAPPI test methods (1994a,b). Kappa number and cellulose content (as α-cellulose) were determined by the TAPPI test methods (TAPPI T 236 cm-85 and TAPPI T 203 om-93) 3 times. Lignin and hemicellulose contents were calculated as: Lignin content (%) = 0.13*K*; Hemicellulose content (%) = 100 – cellulose (%) – lignin (%), where, *K* is the Kappa number (unit less).

2.2.4. Preparation of developed kenaf and bacterial cellulose

Developed bacterial cellulose (DBC) and kenaf cellulose (DKF) were obtained in different yields from the hydrolysis of each cellulose material in different concentrations of hydrochloric acid followed by treatment with 10% sodium hydroxide as shown in Table 1. By this method, the produced cellulose (DBC or DKF) is rapidly dispersed into the solution forming homogeneous gel. The precipitation of the gel with ethanol is critical to the preparation of the developed celluloses from BC (DBC) and Kenaf (DKF). The use of water instead of ethanol converts the gel into a colloid, which was difficult to process.

2.2.5. Microcrystalline cellulose characterizations

2.2.5.1. Water content. The water content of the materials was determined using a Mettler drying apparatus that measures weight loss of sample with an initial weight of two grams at 115 °C for 10 min.

2.2.5.2. Bulk and tap densities. An appropriate amount of the sample was poured in a 50 ml graduate cylinder. The cylinder was lightly tapped twice to collect all the powder sticking on the wall of the cylinder. The volume was then recorded directly from the cylinder and used to calculate the bulk density according to the relationship: density = mass/volume. For tap density, the cylinder was tapped 500 times using a Vankel tap density analyzer. The volume of the sample was then recorded and used in the calculation.

2.2.5.3. Porosity. The porosity of the test powders was determined using the equation $\varepsilon = (1 - \rho_{tap}/\rho_{true})100$, where ε, ρ_{tap}, and ρ_{true} are porosity, tap density, and true density of the powder, respectively.

2.3. Preparation of tablets

Tablets of DKF, DBC and Avicel PH 101 each weighting 200 mg were prepared on a carver hydraulic press at different compression pressures, ranging from about 15 to 155 MPa, using a 13-mm diameter die and flat-face punches and a dwell time of 40 s.

2.4. Fourier transform infrared (FTIR) spectroscopy

Thin samples of the DBC and DKF were prepared according to Kai and Keshk (1998). FTIR spectra were obtained with a Nihon FTIR-480 spectrometer. The FTIR spectra were recorded using about 100 scans with a resolution of 0.5 cm^{-1} for each measurement.

2.5. Wide angle X-ray diffractometry

Thick samples of DBC and DKF were prepared according to Kai and Keshk (1999). The reflect gram of the samples was recorded at room temperature with RIGAKU PRINT 2200V series using Ni-filtered Cu K_α radiation ($\lambda = 1.54$ Å). The operating voltage and current were 40 kV and 30 mA, respectively. Crystallinity index (C.I.) was calculated from the reflected intensity data using the Segal, Creely, Martin, and Conrad method (1959) according to the following equation:

$$\text{C.I.} = \frac{I_{020} - I_{am}}{I_{020}}$$

I_{020} is the maximum intensity of the lattice diffraction and I_{am} is the intensity at $2\theta = 18^\circ$.

2.6. Morphological analysis

Scanning electron microscopy was used to examine the microscopic structure and the surface morphology of the prepared cellulose. For SEM measurements bacterial cellulose membrane was harvested after two days to be more clearly visible. The instrument used for morphological observation was N-2250 from Hitachi Ltd., Tokyo, Japan.

2.7. Solid state ^{13}C NMR spectroscopy

CP/MAS ^{13}C NMR spectra were recorded (at 292 ± 1 K) on a Joel CMX-300 instrument operating at 7.0 T. A double air-bearing probe and a zirconium oxide rotor were used. The MAS rate was in the 4–5 kHz range. Acquisition was performed with a standard CP pulse sequence using a 5 μs proton 90 pulse, a 1200 μs contact pulse and

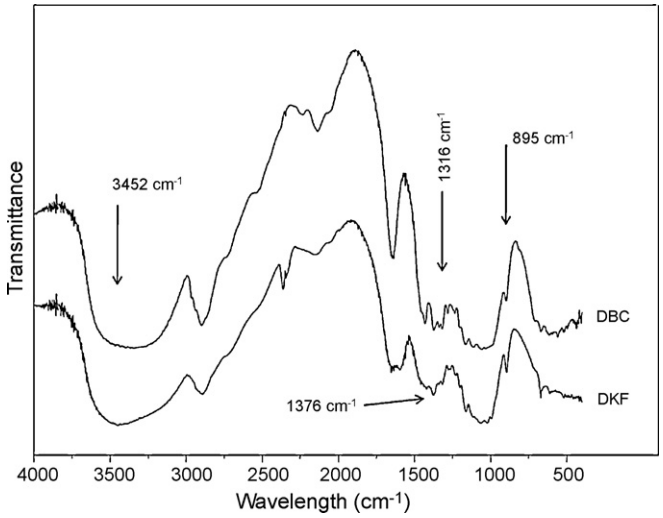


Fig. 1. FT-IR spectra of developed cellulose from *Gluconacetobacter xylinus* and kenaf.

3 s delays between repetitions. Adamantine was used as an external standard for the chemical shift scale relative to tetramethylsilane.

2.8. Themogravimetric analysis

Themogravimetric analysis (TGA) was conducted using a TGA-50 thermal analyzer (Shimadzu, Japan). TG spectra were recorded at a heating rate of 20 °C/min under nitrogen atmosphere.

3. Results and discussions

FTIR spectra of DBC and DKF are depicted in Fig. 1. The two spectra clearly reveal the following differences: (i) the characteristic intermolecular and intramolecular OH stretching vibration band in the spectrum of DKF appeared broader at 3440 cm^{-1} than that of DBC (3452 cm^{-1}); (ii) the peaks at 1376 cm^{-1} and 1316 cm^{-1}, which are associated with the intramolecular hydrogen bonding at the C_6 group and OH in-plane bending vibration, respectively, are less intense in the spectrum of in DKF than those of DBC; and (iii) the absorption band at 895 cm^{-1} in the spectrum of DKF, which is due to anti-symmetric OH out-of-phase stretching vibration is relatively more intense than that of DBC. It has been reported that the intensity of this peak increases with decreasing the crystallinity of the cellulose sample and with changing the crystal lattice from cellulose I to cellulose II (Krassig, 1996). Thus, the higher intensity of this peak seen for DKF compared to that of DBC indicates that the former is of lower crystallinity and contains the cellulose II lattice. Figs. 2 and 3 compare the SEM photographs of DBC, DKF and Avicel PH 101 at different. DBC and Avicel PH 101 appeared to be consisting of aggregated fibers, whereas DKF showed a mixture of non-aggregated and aggregated fiber structure. The particle size of DKF is between 5 and 20 μm while that of DBC is 1–5 μm. The X-ray diffractograms of DBC, DKF and Avicel PH 101 are shown in Fig. 4. The X–ray pattern of DBC and Avicel PH 101 demonstrates reflections that are characteristics of the cellulose I lattice, as indicated by the diffraction peaks at about $2\theta = 15°$,16° and 23° (Kai and Keshk, 1998). Whereas, the X-ray pattern of DKF shows reflections that are characteristics of cellulose II lattice. The X–ray pattern of DKF exhibits diffractions peaks at about $2\theta = 12°$, 20°, and 22° due to 1 1 0, 1 $\bar{1}$ 0, and 0 2 0 planes, respectively (Kai and Keshk, 1998). The calculated crystallinity index values of DBC, DKF and Avicel PH 101 samples were 85%, 70% and 77%, respectively. These differences in the crystallinities are attributed to the different crystal lattices of the cellulose crystal structures. In cellulose I, the chains are arranged in a parallel manner, whereas cellulose II exhibits an anti-parallel arrangement of chains (Krassig, 1996). The different degree of crystallinity is due to the different chain arrangements result in different interchain and intrachain hydrogen bonding networks. Studies showed that cellulose II possesses additional hydrogen bonding between chains at the corners and at the centers of the unit cells (Krassig, 1996).

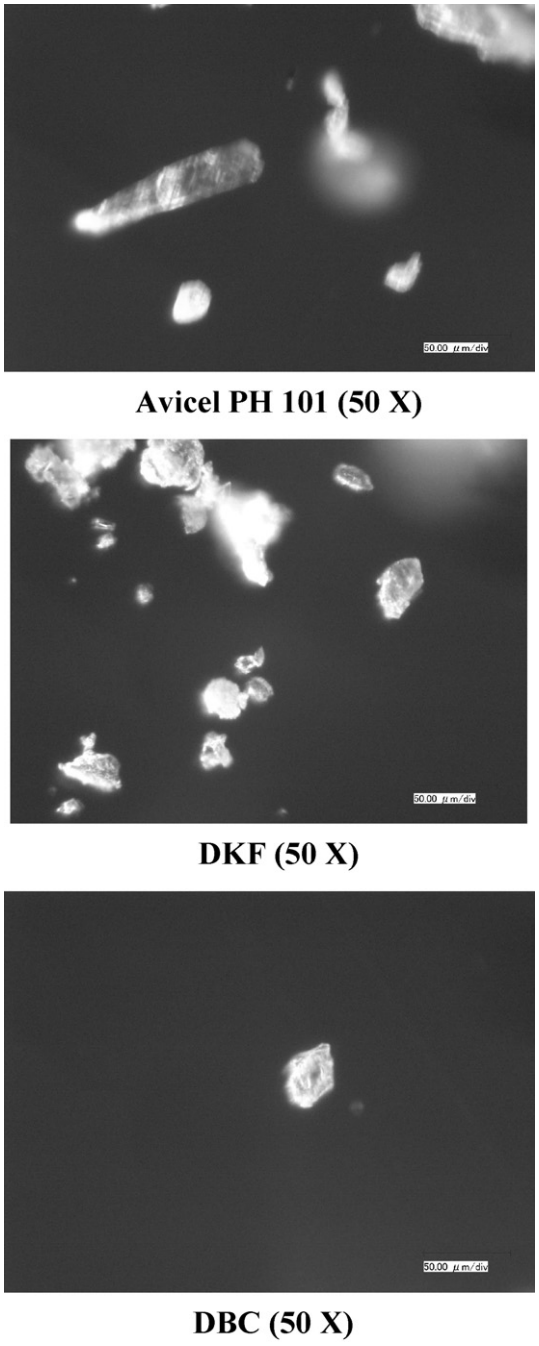
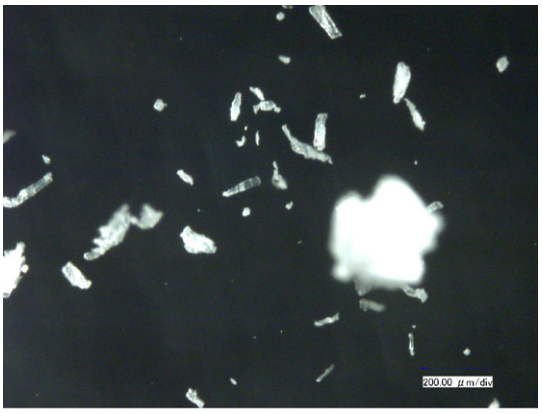
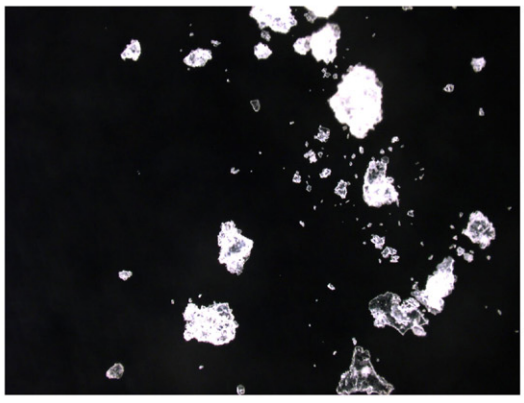


Fig. 2. Scanning electron micrographs (50×) from Avicel PH 101, kenaf and *Gluconacetobacter xylinus*.

The CP/MAS ^{13}C NMR spectra of DKF and DBC are depicted in Fig. 5. The doublet peak of C-1 that appeared at 105.3 ppm and 104.5 ppm and 63.2 ppm that due to C-6 prove the cellulose II lattice (Keshk et al., 2006). Furthermore, the peaks at 89.3 ppm and 84.9 ppm are due to crystal and amorphous regions of C-4. The chemical shifts of 75.4 ppm, 74.5 ppm and 72.7 ppm are attributed to C-2,3,5. The peak at about 84.0 ppm in the spectrum of DKF, is





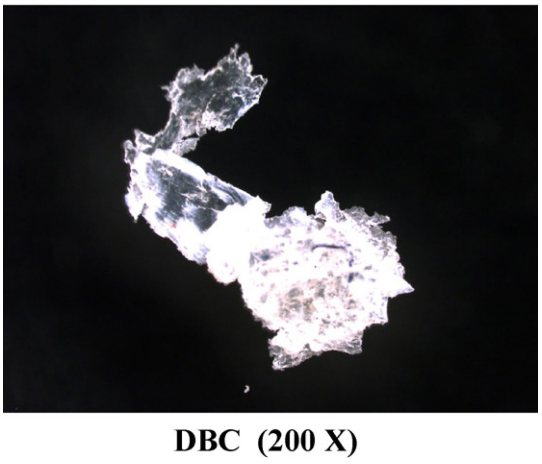


Fig. 3. Scanning electron micrographs (200×) from Avicel PH 101, kenaf and *Gluconacetobacter xylinus*.

less intense in the spectrum of DBC. This may be explained by the highest crystallinty of DBC compared to DKF. The singlet peak of C-1 in the spectrum of DBC appeared at 106.0 ppm and that of C-6 at 65.6 ppm prove the cellulose I lattice (Keshk, 2008). The TGA was conducted in order to assess the thermal stability of DBC and DKF.

The thermal curves of both cellulose materials are shown in Fig. 6. The character of the TGA for the DBC sample points to a very homogenous composition. The TGA results also reveal increased thermal stability for DBC compared to DKF. Moreover, it can be seen that the weight loss of DBC occurred in one step degradation process from about 320 °C to 380 °C, which is mainly due to the decomposition of cellulose. On the other hand, the thermal degradation of DKF occurred in a two step degradation process from approximately 215 °C to 345 °C. Clearly, the peak weight loss temperature of DKF is lower than DBC by 105 °C. These differences in the thermal properties of both cellulose materials are mainly due

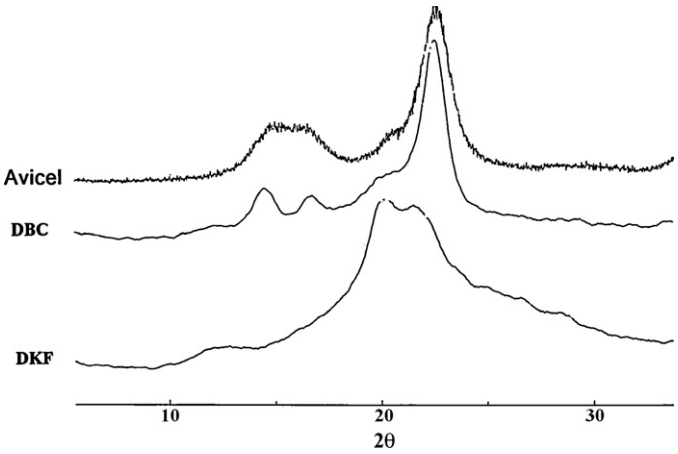


Fig. 4. X-ray pattern of cellulose from Avicel PH 101, *Gluconacetobacter xylinus* and kenaf.

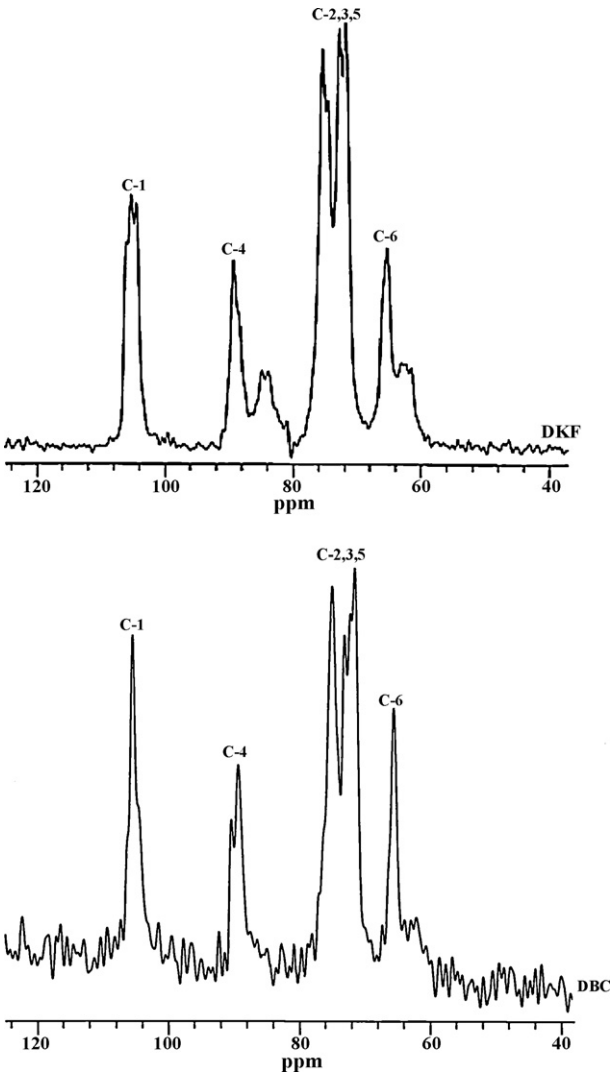


Fig. 5. CP/MAS ^{13}C NMR spectra of cellulose from kenaf and *Gluconacetobacter xylinus*.

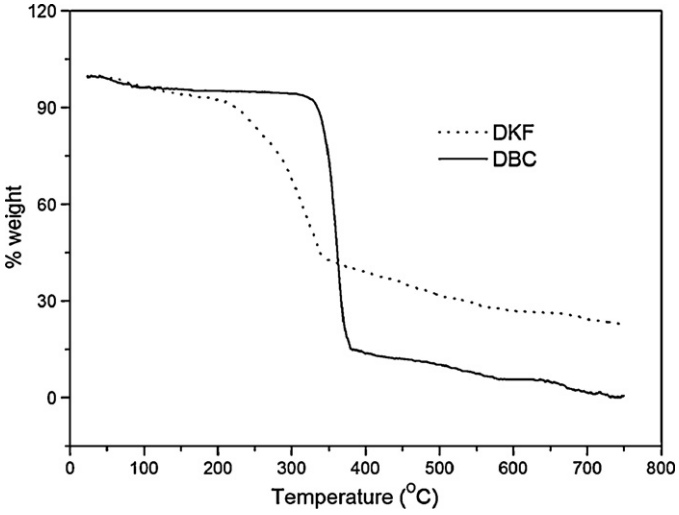


Fig. 6. The TGA curves of DKF and DBC samples heated at 20 °C/min in nitrogen atmosphere.

Table 2
Physical properties of DKF, DBC and Avicel PH-101.

Parameter	DKF	DBC	Avicel PH 101
Degree of crystalinity (%)	70	85	77
Moisture content (%)	4.0	1.6	2.0
Porosity (%)	60	82	75
True density (g/ml)	1.50	1.53	1.56
Bulk density (g/ml)	0.468	0.342	0.321
Tap density (g/ml)	0.534	0.432	0.421

to differences in their degree of crystallinity. This agrees with XRD data (Fig. 4) where DBC exhibits 85% degree of crystallinity and DKF 77%. This revealed a relationship between crystal structure and the thermal degradation of cellulose. A greater crystalline structure required a higher degradation temperature (Yang & Kokot, 1996) and, therefore, DBC degraded at 320 °C whereas DKF at 215 °C.

Powder properties of DKF, DBC and Avicel PH 101 are compared in Table 2. Owing to its non-aggregated, fibrous structure, DKF is less porous and shows higher bulk and tap densities compared to those of DBC and Avicel PH 101. The moisture content of DKF was 4.0% while that of DBC was only 1.6%. The higher moisture content observed in DKF is due to its lower crystallinity which causes more hydroxyl groups to be accessible for interaction with water molecules beside to different cellulose chain arrangements and, consequently, the hydrogen bonding network.

4. Conclusion

This study showed cellulose that derived from both BC and Kenaf, when soaked in sodium hydroxide solution and subsequently precipitated with ethanol /water result in materials that can be compressed into tablets without the need of a binder. The developed DBC is predominantly a fibrous material consisting of the cellulose I lattice. Compared to DKF, DBC is more dense and less ductile. The higher density of DBC is important in tabletting because the volume of the die-fill would be correspondingly decreased. In conclusion, the results presented show that DBC has the potential to be used as a direct compression excipient.

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