Biomass Conversion and Biorefinery (2023) 13:311–320
https://doi.org/10.1007/s13399-020-01119-9

ORIGINAL ARTICLE

Extraction of lignocellulosic constituents from cow dung: preparation and characterisation of nanocellulose

Shivani Puri[1,2] · Sarthak Sharma[3] · Avnesh Kumari[3] · Mohit Sharma[1,2] · Upendra Sharma[1,2] · Sanjay Kumar[2,3]

Received: 10 August 2020 / Revised: 22 October 2020 / Accepted: 29 October 2020 / Published online: 26 November 2020

Abstract

Continuous growth of the livestock population over the years has led to the generation of huge livestock waste. Inadequate management of animal waste and its poor disposal poses a serious public health issue and also leads to environmental pollution. In the recent decade, the rational use of lignocellulosic biomass towards preparation of bioproducts such as biofuels and biotextile has come up as a promising alternative for the efficient utilisation of animal waste biomass. Hence, the current study is focused on the efficient utilisation of an abundant source of lignocellulosic constituents, i.e. cow dung by the extraction and estimation of cellulose, lignin, and hemicellulose, respectively by an optimised kraft pulping method. The extracted cellulose was evaluated for the degree of polymerisation and was further used for the preparation of nanocellulose which has a wide range of applications. Cellulose and nanocellulose so obtained were morphologically and spectroscopically characterised by SEM-EDX, CHNS elemental analysis, TEM, and FT-IR techniques. Further, nanocellulose was also evaluated for its physical properties such as crystallinity index and zeta potential, which revealed their good crystallinity and excellent particle stability profile. Hence, this study is an innovative approach towards the valorisation of cow dung waste biomass into useful biomaterial, i.e. nanocellulose.

Keywords Lignocellulosic biomass · Cow dung · Cellulose · Nanocellulose · Morphological and spectroscopic analysis

Abbreviations

LB	Lignocellulosic biomass
KP	Kraft pulping
NC	Nanocellulose
α-NC	Alpha-nanocellulose
DP	Degree of polymerisation
CI	Crystallinity index

✉ Mohit Sharma
mohit@ihbt.res.in

✉ Upendra Sharma
upendra@ihbt.res.in

✉ Sanjay Kumar
sanjaykumar@ihbt.res.in

[1] Chemical Technology Division, CSIR-IHBT, Palampur 176061, India

[2] Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, U.P. 201002, India

[3] Biotechnology Division, CSIR-IHBT, Palampur 176061, India

1 Introduction

Livestock sector is one of the fastest growing sectors globally. In 2019, the global livestock population accounts for about 989.03 million heads [1]. Developing economy like India accounts for 186 million in the production of large cattle, and it also holds the position of home to the highest population of cows in the world in 2019 [1]. The production of livestock on an industrial scale leads to simultaneous production of huge amount of dung which poses a serious threat to environment [2]. Animal dung waste management is a major challenge faced by the dairy industry and public health institutions globally. Cow dung waste is one of the major sources of animal waste biomass from the bovine dairy industry [3]. Although cow dung is a very well-explored source as manure and possesses high socio-economic value due to its important role in the village economy in the developing economies, continuous growth in the livestock population over the years in the bovine dairy industry has led to the generation of huge underutilised cow dung waste [4]. The annual generation and improper waste management of around 2600 million tons of dung are ultimately leading to environmental pollution by greenhouse gas release and contamination of ground water and streams [5,

6]. Hence, cow dung is an abundant unexploited source which presently due to its improper waste management has emerged as a matter of environment concern. Therefore, research and development of novel eco-friendly and innovative processes is the need of the hour for the efficient utilisation of cow dung waste biomass for several value-added products.

The utilisation of abundant renewable biomass for the sustainable production of various value-added products has grabbed greater attention in the past two decades. Lignocellulosic biomass (LB) is one such renewable and abundant source which can substitute synthetic polymers due to its eco-friendly properties. Cellulose, hemicellulose, and lignin are the three main components of the LB. There are numerous reports which highlight the importance of lignocellulosic-rich plant materials for the extraction and development of cellulose-based bioproducts. Some of the notable sources such as bamboo, sugarcane bagasse, rice husk, waste paper, fruit waste, and other fibrous plants such as kenaf core, coconut coir, bagasse fibres, maize, and sugarcane has been well explored for the production of cellulose-based biomaterials such as nanocellulose (NC), microcrystalline cellulose, composite material, boards, biofuels, biopaper, bioadsorbents, and regenerated cellulose [7–11]. Hence on through survey of literature, we found that most of the studies for the extraction of cellulose have been performed using fibrous plant sources and agricultural waste biomass. On the other hand, cow dung waste biomass which is an economical and abundantly available LB resource is unexplored and can act as a potential source of lignocellulosic constituents such as cellulose.

Cow dung waste is the undigested residue of consumed grass or grains defecated by bovine animals. It is a mixture of faeces and urine in the ratio of 3:1. Cellulose, hemicellulose, and lignin are the main constituents of cow dung waste. It is estimated that 40% of cow dung is undigested cellulose [12]. Cellulose is a linear polymer comprising of several hundred to thousands of β $(1 \rightarrow 4)$ linked *D*-glucose units and exists in three different forms, i.e. alpha, beta, and gamma cellulose, which are distinguished on the basis of solubility and its degree of polymerisation. Cow dung also contains 24 different minerals, viz. nitrogen, potassium, phosphorus (NPK—3:2:1) along with trace amount of sulphur, iron, magnesium, copper, manganese, and cobalt. It also has a high level of NH_3, organic matter and harbours a diversity of microbes. The lignocellulosic composition of cow dung manure reported in the literature as 1.6–23.5% cellulose, 1.4–12.8% hemicellulose, and 2.7–13.9% lignin [13]. Hence, the % cellulose composition of cow dung makes it a suitable feedstock for the extraction of cellulose for the generation of cellulose-based biomaterials.

Cellulose-based biomaterials such as nanocellulose, microcrystalline cellulose, biofuels, biopaper, bioadsorbents, and regenerated cellulose have recently emerged as a potential alternative to their synthetic counterparts due to their eco-friendly nature. Out of these, nanocellulose (NC) is an important biomaterial which has huge applications in pharmaceuticals, filtration, implants, hydrogel, packaging, automotive, electronics, construction, paint, and paper [14, 15]. NC is the term used for the cellulose fibres which has at least one of the dimensions in nanoscale [16]. Depending on the size and source of origin, NC has been classified mainly into two types, i.e. cellulose nanofibrils and bacterial cellulose. NC is rod-like particles obtained after the disintegration of cellulose fibres with acidic hydrolysis. NC has many interesting properties like high aspect ratio and high crystallinity that could be easily tailored by altering the reaction conditions such as concentration of acid, reaction time, and temperature. Surface modification could be easily achieved due to the abundance of hydroxyl groups on its surface. The conversion of cellulose into NC can be achieved by various physical, chemical, and enzymatic procedures, depending on the size of the NC required [17, 18]. Preparation of NC from sisal, banana rachis, kapok, coir, and pineapple leaf has been very well reported [19]. The preparation of NC from different biomass sources is one such domain which has gained huge interest worldwide, but it remains unexplored in the domain of animal waste biomass which is a huge untapped resource.

Thus, we aim to study the extraction of cellulose for the preparation of cellulose-based biomaterials, i.e. α-NC from cow dung waste biomass. Hence, this study will help to reduce the environmental impact of cow dung waste by exploring its potential for the valorisation into value-added products.

2 Materials and methods

2.1 Raw material collection

The fresh cow dung sample was collected from Dr. GC Negi College of Veterinary and Animal Science, Palampur, H.P. The sample was dried under sunlight to avoid fungal growth and kept in a hot air oven at 105 °C for 30 min to disinfect the sample. The dried sample was then powdered and sieved to coarse particle size.

2.2 Extraction of cellulose from cow dung

2.2.1 Pre-hydrolysis of cow dung

A total of 4 kg of dried and powdered cow dung was pre-hydrolysed with 0.3% H_2SO_4 in a 15-L stainless steel digester rotating at 1 rpm and maintained at 120 °C for 30 min. The solid-to-liquid ratio was taken as 1:10 to ensure a homogenous mixture. After pre-hydrolysis, the pulp was filtered using a cloth filter and washed several times with distilled water to completely remove the acid residues. Subsequently, the pulp was dried in the hot air oven at 50 °C. The filtrate was kept for the estimation of hemicellulose content present in the sample. The percentage yield of the pre-hydrolysed sample was

calculated on dried weight basis of pulp using the formula % yield = weight of dried pre-hydrolysed pulp sample/weight of dried cow dung sample * 100.

2.2.2 Optimisation of the kraft pulping method for the extraction of cellulose from cow dung

The kraft pulping (KP) process is extensively used for the extraction of cellulose from fast-growing wood species in the paper and pulp industries [20]. KP involves the treatment of alkali (NaOH) and sulphide (Na_2S) to the pre-hydrolysed pulp. Concentration of chemicals required for this process depends upon the nature of sample. Since cow dung biomass is partially digested cellulose biomass, hence it requires less chemical treatment as compared to the wood biomass. Thus, alkali, sulphide concentration, temperature, and time required in the KP of cow dung biomass were optimised in order to achieve good yield with minimum amount of chemical treatment, temperature, and time. The optimisation of KP was performed in a stepwise manner for their pulp production efficiency in terms of percent yield of cellulose and colour of pulp. Initial concentrations of NaOH (10%, 15%, and 20%) were varied keeping the concentration of sulphide constant, i.e. 5%, at 120 °C for 30 min. After NaOH concentration optimisation, different concentrations of Na_2S (5%, 10%, and 15%) were analysed. Similarly, different time intervals (30 min and 60 min) and temperature (120 °C and 160 °C) conditions were also optimised in order to achieve maximum yield. The optimised conditions for the KP process were found to be 15% NaOH and 10% Na_2S at 160 °C for 60 min. The % yield, colour of cellulose pulp with varied values of alkali, sulphide concentration, temperature, and time obtained from different optimisation experiments of the kraft pulping process have been mentioned in Table 1.

2.2.3 Extraction of cellulose

The kraft pulping of the dried pre-hydrolysed sample was carried out in a 15-L stainless steel digester according to the optimised set of conditions. The digested cellulose pulp was filtered using a cloth filter and washed with distilled water to completely remove the chemical residues present in the pulp. The washed pulp was dried and then bleached to obtain white cellulose pulp. The four different stages of bleaching involved are discussed below:

- Hypochlorite bleaching—the cellulose pulp with consistency of 15% was treated with a homogenous mixture of calcium hypochlorite (0.7%, w/v) and calcium hydroxide (0.3%, w/v) and was agitated for 3 h at 60 °C. After completion, the pulp was cloth filtered and washed with distilled water until the complete removal of chemical residue. The washed pulp was dried in the hot air oven at 50 °C.
- EDTA bleaching—the hypochlorite-bleached cellulose pulp was treated with 2% EDTA solution for 1 h at 55 °C. After this, the pulp was cloth filtered, washed with distilled water, and dried in the hot air oven at 50 °C.
- Hydrogen peroxide bleaching—the EDTA-bleached cellulose pulp was treated with a homogenous mixture of Na_2HPO_4 (0.5%) and KH_2PO_4 (0.3%) along with equal proportion of H_2O_2 for 1 h at 85 °C. Similarly, the bleached pulp was then cloth filtered, washed with distilled water, and dried in the hot air oven at 50 °C.
- EDTA bleaching—again the 2nd EDTA bleaching step was performed with 2% EDTA solution for 1 h at 55 °C. After bleaching, the pulp was cloth filtered, washed with distilled water, and dried in the hot air oven at 50 °C.

The percentage yield of the extracted cellulose pulp (USSP02) was determined on a dried weight basis of pulp using the equation % yield = weight of dried bleached pulp/weight of dried pre-hydrolysed pulp * 100.

3 Preparation of α-NC

Hydrolysis of α-cellulose (αUSSP02) was done using sulphuric acid (H_2SO_4). Briefly, 4 g of sample was added to 40 ml of distilled water and continuously stirred at 45 °C until

Table 1 Optimisation of % NaOH and % Na_2S, temperature, and time for the kraft pulping process

Sample code	NaOH (%)	Na_2S (%)	Temp. (°C)	Time (min)	% yield of cellulose pulp	Colour of cellulose pulp
USSP1	10	5	120	30	6.3	Light brown
USSP2	15	5	120	30	7.4	Light brown
USSP3	20	5	120	30	7.9	Light brown
USSP4	15	10	120	30	11.2	White
USSP5	15	15	120	30	9.3	White
USSP6	15	10	160	30	8.7	White
USSP7	15	10	160	60	13.0	White

proper slurry of cellulose pulp was formed. Further, 40 ml of 65% H_2SO_4 was added to this slurry. After 1 h of acidic treatment at 45 °C, suspension was diluted with double-distilled water in order to quench the reaction. The process was then followed by centrifugation and dialysis for the complete removal of acid. The schematic flow chart for the extraction of cellulose and preparation of α-NC from cow dung is shown in Fig. 1.

4 Estimation of lignocellulosic constituents and their parameters

4.1 Hemicellulose content

The hemicellulose content was measured according to the TAPPI T249 cm-09 standard method by gas chromatography–flame ionisation detection (GC-FID) analysis. The pre-hydrolysed filtrate was hydrolysed using 3 ml of 72% H_2SO_4 for 1 h at 30 ± 0.5 °C on a water bath with occasional stirring. After this, the sample was neutralised and neutralised sugars were reduced to alditols by treatment with sodium borohydride. The reduced alditols were acetylated with acetic anhydride and H_2SO_4. Lastly, the acetylated sample was used for the GC-FID analysis and calculations were done on the basis of retention time and area of peaks.

4.2 Alpha-cellulose content

The α-cellulose content was measured according to the TAPPI T203 cm-99 standard method. Suitable amount of moisture-free cellulose pulp (USSP02) was weighed and dispersed in 17.5% NaOH for 30 min at 25 ± 0.2 °C. Then, equivalent amount of distilled water was added into the sample and stirred for another 30 min at 25 ± 0.2 °C. After a total interval of 1 h, pulp was filtered, was washed with dilute alkali and water, and was further oven dried at 50 °C. The % yield of α-cellulose was calculated on a dry weight basis. The α-cellulose (αUSSP02) so obtained was further used in the preparation of α-NC.

4.3 Acid-insoluble lignin content

The lignin content was measured according to TAPPI T222 om-02. The black liquor obtained from kraft pulping exhibits the pH of range 12–13 that was used for the estimation of acid-insoluble lignin. The black liquor was treated with dilute H_2SO_4 (72%) and stirred on a magnetic stirrer for 2 h. The sample was diluted with water up to 3% of sulphuric acid concentration to extract maximum amount of lignin at low pH value. The solution was boiled for 4 h and kept undisturbed overnight as it leads to formation of precipitates. The precipitates were washed, filtered, and dried and % yield of lignin was determined [21, 22].

4.4 Evaluation of viscosity, degree of polymerisation, and molecular weight

The viscosity measurement was done according to SCAN-C15:62 guidelines. Cellulose pulp (USSP02) was dissolved in 1 M cupriethylenediamine (CED) solution and viscosity was measured by using an Ostwald viscometer. The viscosity

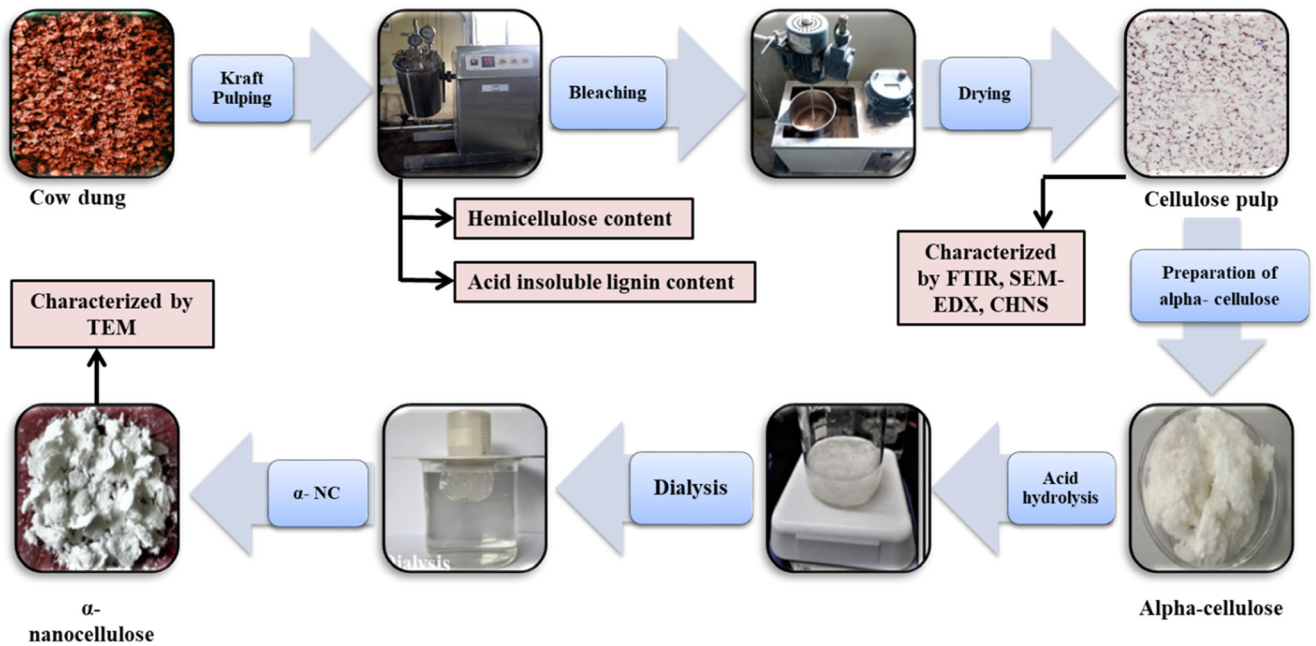

Fig. 1 Schematic view of extraction of cellulose and preparation of nanocellulose from cow dung using different chemical and mechanical treatments

so obtained was used for the calculation of intrinsic viscosity. The degree of polymerisation (DP) of extracted cellulose was then calculated according to the Mark–Houwink–Sakurada equation, i.e. $P^{0.90} = 1.65[\eta]/\text{ml g}^{-1}$ where P is indeterminate average DP and η is the intrinsic viscosity [23–25]. Molecular weight of the extracted cellulose was calculated from the DP by multiplying it with the molecular weight of anhydroglucose unit.

4.5 Evaluation of ash content

The ash content of cellulose pulp was measured according to the TAPPT T211 om-02 standard method. A total of 1 g of moisture-free cellulose pulp (USSP02) was taken in pre-weighed crucible and charred. After charring, crucible was kept in muffle furnace at 525 ± 25 °C for 60 min. On completion, sample was cooled and kept in a desiccator and its ash content was determined [26].

5 Characterisation of cellulose and α-NC

5.1 Fourier transform infrared spectroscopic analysis

The Fourier transform infrared (FT-IR) spectra of the cow dung sample and extracted cellulose pulp sample (USSP02) were recorded using a Shimadzu IR Prestige-21 equipped with ZnSe single-reflection ATR accessory in the wavenumber range of 4000–400 cm^{-1}. A total of 5 mg of oven-dried cellulose and cow dung samples were used to record IR spectra with a 4 cm^{-1} resolution and 40 scans.

5.2 Scanning electron microscopy–energy-dispersive X-ray (SEM-EDX) analysis

To study the surface morphology of the cellulose, SEM imaging of the cellulose pulp (USSP02) was done. The dried sample was mounted on a specimen stub and coated with gold using sputter coating unit (E1010, Hitachi), and images were captured on desired magnification at 15KV (S-3400 N Hitachi, Japan). Elemental composition of the cellulose sample was also determined by EDX for the characterisation of extracted cellulose pulp.

5.3 CHNS elemental analysis

The CHNS elemental analysis of cellulose pulp was also performed using the CHNS analyser vario MICRO cube (Elementar, Germany). A total of 2–3 mg of sample was wrapped in a tin foil and loaded into the elemental analyser where it undergoes combustion in the combustion tube operating at 1150 °C. Gases produced after combustion of organic element were analysed for estimation of carbon and hydrogen present in extracted cellulose.

5.4 Transmission electron microscopy

TEM (Tecnai, Twin 200 KV, FEI, Netherlands) was used to obtain the micrographs of the α-NC at an accelerating voltage of 200 kV. The sample was prepared by drop casting a dilute aqueous suspension (0.1 wt%) of α-NC onto a carbon-coated copper grid and was allowed to dry for 10 min. Further, staining with 3 wt% solution of uranyl acetate was done in order to enhance the contrast in TEM micrographs.

5.5 Zeta potential measurement

The surface charge of NC was analysed by zeta potential measurements so as to see their stability in the solution. Laser Doppler microelectrophoresis is used to measure zeta potential. The dilute aqueous suspension (0.1 wt%) of NC was characterised by Zetasizer Nano ZS (Malvern Instruments Ltd.).

5.6 Powder X-ray diffraction analysis

X-ray diffraction was done to check the crystallinity index of the α-cellulose (αUSSP02) and α-NC (αUSSPNC02). Sample was analysed using the Rigaku SmartLab 9-kW rotating anode X-ray diffractometer at room temperature using monochromatic CuKα radiation ($\lambda = 0.154$ nm) source in the continuous scan mode at 2 range from 10 to 80°. The diffractogram was generated using Origin pro8.5, and the crystallinity index of the sample was determined as percentage of ratio of area of crystalline peak to the total area under the curve [27].

6 Results and discussion

6.1 Optimisation of the kraft pulping process for the extraction of cellulose from cow dung

The lignocellulose exists in a very compact structure where cellulose is wrapped within the matrix of hemicellulose and lignin. To extract cellulose, several pre-treatments are necessary which leads to specific chemical and physical changes in the cellulose, hemicellulose, and lignin component of the biomass. Pre-hydrolysis is required to remove the lignin and hemicellulose from the cow dung. Pre-hydrolysis step leads to dissolution of hemicellulose and also lignin to a lesser extent. Pre-hydrolysis helps in extraction of pure cellulose as it minimises hemicellulose impurities [28, 29]. Hence, the acid pre-hydrolysis was initially performed to remove the hemicellulose and the % yield of pre-hydrolysed sample was found to

be 31.74%. After the pre-hydrolysis step, optimisation of the KP method for the cow dung sample was done. NaOH is used to maintain alkaline pH of the solution and also to promote swelling of cellulose fibres. The digestion interval, temperature, and sulphide concentration play an important role in preparation of delignified cellulose. Hence, these parameters were optimised for the KP method. During alkali (% NaOH) optimisation, we found that there is a significant difference in % yields of cellulose pulp with increasing concentration from 10 to 15%, whereas no notable difference was observed between 15 and 20% NaOH; hence, 15% NaOH was opted as the best alkali concentration. On increasing sulphide concentration, there is an increase in % yield of cellulose pulp. Hence 10% Na_2S is chosen as the best concentration of sulphide. Similarly, higher temperature at 160 °C and increased time interval of 1 h also showed better % yield as compared to the lower conditions. Ultimately, the optimised conditions for extraction of cellulose pulp from cow dung were found to be 15% NaOH and 10% Na_2S at 160 °C for 1 h. From the study, we found that the digestion parameters required for KP of cow dung biomass were lower in comparison to wood biomass which is reported in the range of 15–21% NaOH and 24–35% Na_2S [20]. The % yield of the cellulose obtained from the kraft pulping process was found to be 11.1% which is less as compared to other plant biomass sources, i.e. 49.3% in bamboo and 55.75% in Iranian sugarcane bagasse [30, 31].

6.2 Estimation of hemicellulose, α-cellulose, acid-insoluble lignin, and ash content

Lignocellulosic constituents obtained from waste biomass are renewable and abundant resource for the bioconversion into value-added products. The pulping process leads to extraction of all basic components such as hemicellulose, lignin, and cellulose which can be converted to useful by-products. The lignocellulosic composition of cow dung was determined using standard TAPPI procedures. The α-cellulose content (8%) of the cellulose pulp obtained from cow dung is found to be lower in comparison to the wood species [32]. In hemicellulose sugars, arabinose was found to be the main sugar present in cow dung. The hemicellulose content was concluded on the basis of retention time in GC-FID analysis. The ash content is indicative of the amount of inorganic residues single or in combination with other residues. The residues can be from chemicals used during processing, mineral matter in pulp, or other added materials. Thus, the ash content of cellulose was determined on a dry weight basis and it was found to be 7.56%. The % yields of cellulose pulp, α-cellulose, hemicellulose, and acid-insoluble lignin content are listed in Table 2. The lignocellulosic composition profile of the cow dung provides a clear indication that it can be used as a potential feedstock in the development of various value-added materials from cellulose and lignin. It can also be utilised in preparation of LB-derived platform chemicals using biorefining technologies [33].

6.3 Evaluation of viscosity, degree of polymerisation, and molecular weight

Cellulose is a linear polymer of several units of D-glucose. The number of monomers attached affects physical properties such as solubility, density, crystallinity, polarity, and mechanical property like tensile strength. The viscosity of the cellulose solution is the indicative of the degree of polymerisation and molecular weight of the compound [34]. Hence, the molecular weight and DP of the cellulose pulp were determined from the intrinsic viscosity which is obtained from actual viscosity of cellulose solution in CED. The intrinsic viscosity, DP, and molecular weight of the extracted cellulose from cow dung are listed in Table 3. The lower DP value indicates more solubility of cellulose pulp with lesser tensile strength of cellulose fibres.

6.4 Characterisation of cellulose and α-NC

6.4.1 Fourier transform infrared spectroscopic analysis

The FT-IR spectrum of cow dung and cellulose extracted from the cow dung is shown in Fig. 2. In the spectrum, absence of any absorbance in between 1600 and 1510 cm^{-1} signifies complete absence of lignin from the cellulose pulp whereas peak at 1512 cm^{-1} in the cow dung shows presence of lignin. The absorption at the wavenumbers 3332 cm^{-1} and 3375 cm^{-1} is due to

Table 2 α-Cellulose, hemicellulose, lignin, and ash content and % yield of cellulose extracted from cow dung

Parameters	Sample code—USSP02*	Methodology
% yield of cellulose pulp	11.1%	Optimised kraft process
α-Cellulose	8%	TAPPI T203 cm-99
Hemicellulose (cow dung)	2.261%	TAPPI T249 cm-09
Acid-insoluble lignin (cow dung)	40%	TAPPI T222 om-02
Ash	7.56%	TAPPT T211 om-02

*USSP02 represents the cellulose extracted from cow dung by the optimised KP method

Table 3 Intrinsic viscosity, DP, and molecular weight of cellulose extracted from cow dung

Parameter	Sample code—USSP02*	Standard method
Intrinsic viscosity	57	SCAN-C15:62
DP	155.089	-
Molecular weight	25,110	-

*USSP02 represents the cellulose extracted from cow dung by the optimised KP method

stretching vibrations of O-H groups and at 2895 cm^{-1} and 2860 cm^{-1} due to symmetric C-H stretching vibrations in both the samples. The peak at 2918 cm^{-1} is due to asymmetric stretching vibrations in the cow dung sample. Stretching vibration at 2337 cm^{-1} is for CO_2 and at 1639 cm^{-1} and 1635 cm^{-1} is due to bending of absorbed water in both samples as concluded from Sun et al. [35]. The band at 1321 cm^{-1} is due to C-O and C-C vibrations deduced from a report by Pastorova et al. [36]. The absorption peak at 1157 cm^{-1} corresponds to the presence of C-O antisymmetric bridge stretching along with the vibration at a wavenumber range of 1076–1022 cm^{-1} which signifies vibrations of C-O-C pyranose ring skeletal. The band at 896 cm^{-1} is due to β-glycosidic linkage among different glucose units present in cellulose as concluded from Pappas et al. [37]. Thus, the presence of cellulose in the sample was characterised by the presence of relevant peaks in the FT-IR spectrum.

6.4.2 Scanning electron microscopy/Energy-dispersive X-ray analysis

The scanning electron microscopy is used for studying the surface topography and diameter of cellulose fibres. The SEM image of cellulose pulp extracted from cow dung is shown in Fig. 3a, b. SEM revealed the surface topography of fibres after chemical pre-treatment. The size distribution is obtained using the ImageJ software. It is observed that fibres showed a narrow size distribution with an average size of 10–15 µm. Energy-dispersive X-ray analysis is performed in order to confirm the existence of basic elements present in the cellulose. EDX is a reliable and easy technique for the

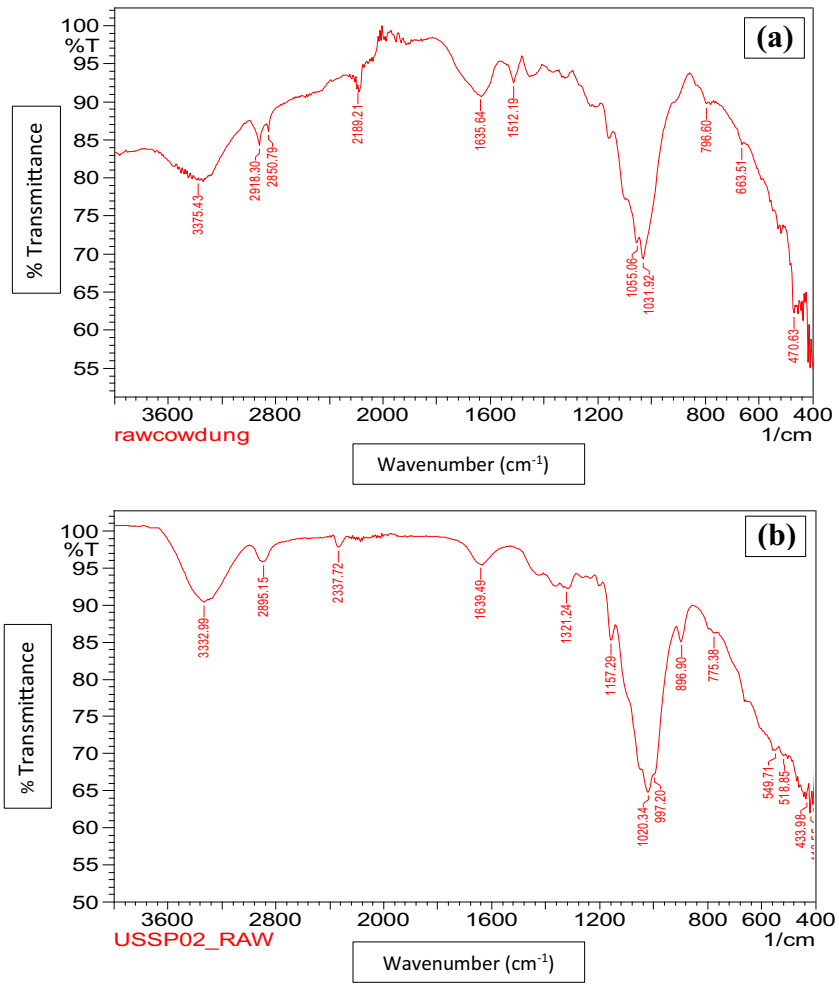

Fig. 2 The FT-IR spectrum of **a** raw cow dung and **b** cellulose extracted from cow dung (USSP02)

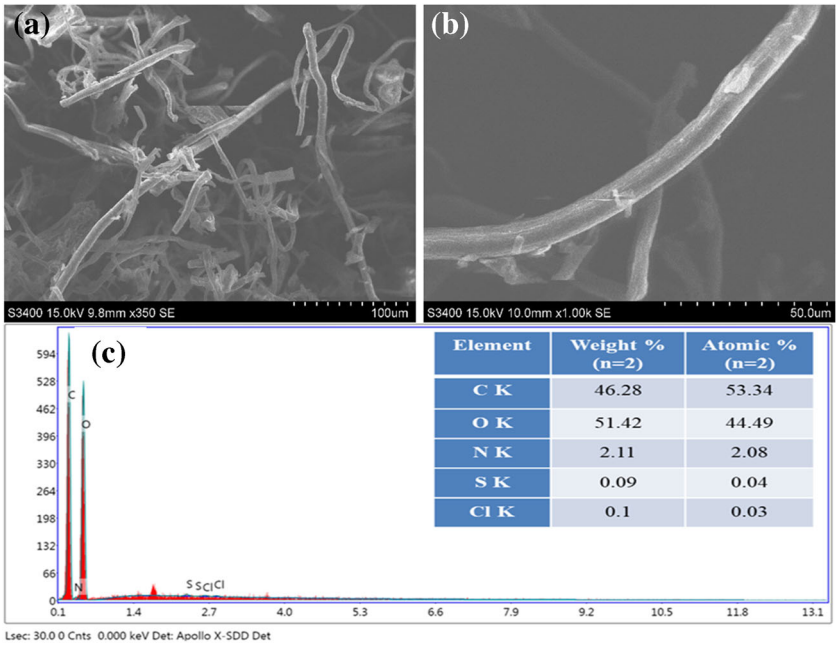

Fig. 3 SEM images of cellulose pulp extracted from cow dung at **a** 100 μm and **b** 50 μm and **c** EDX spectra of cellulose

characterisation of structure of compound. The EDX results confirm the presence of 46.28% carbon and 51.42% oxygen in the extracted cellulose from cow dung (Fig. 3c). The observed elemental composition is found to be in agreement with natural cellulose elemental composition, i.e. 44.44% carbon and 49.39% oxygen.

6.4.3 CHNS elemental analysis

The elemental analysis by combustion is a rapid and efficient technique of determining organic elements (CHNS) present in a sample. The data of elemental analysis showed that cellulose extracted from cow dung contains 36.22% carbon and 5.33% hydrogen. The composition of carbon and hydrogen was found to be comparable to the elemental composition of natural cellulose, i.e. 44.44% carbon and 6.17% hydrogen, which confirms the presence of cellulose in the sample.

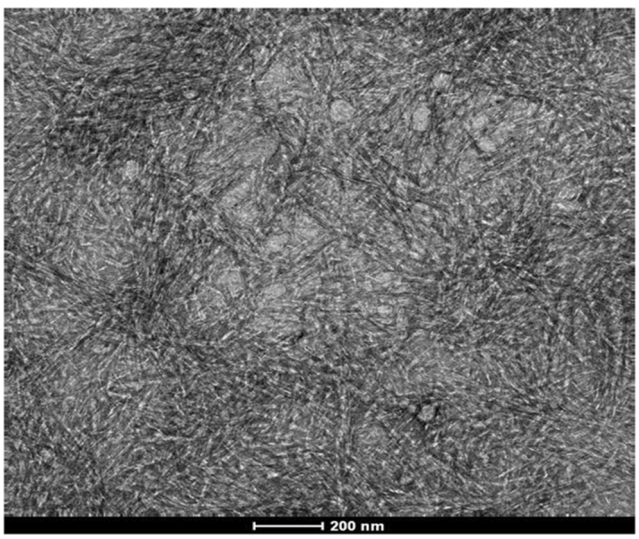

Fig. 4 Transmission electron micrograph of α-NC

6.4.4 Transmission electron microscopy

Transmission electron microscopy was used to study the structure of nanocellulose. The TEM micrograph of α-NC prepared from α-cellulose extracted from cow dung is shown in Fig. 4. The dimensions and aspect ratio (length:width) of α-NC isolated under controlled conditions were studied by the analysis of the TEM images resulting in an average width of 30 nm with an average length of 400 ± 100 nm. The aspect ratio of isolated nanocellulose was 10. Size of isolated NC is comparable to the size of NC obtained from different plant species [38].

6.4.5 Zeta potential measurement

The surface charge of α-NC analysed by dynamic light scattering and zeta potential measurements gave high negative values of zeta potential (− 37.53 mV) which occurs as a result of functionalisation of α-NC with the anionic sulfonic groups introduced during the sulphuric acid hydrolysis step during the preparation of α-NC from cellulose. The ideal value of zeta potential should be less than − 30 mV or higher than 30 mV or 25 mV. The greater zeta potential so obtained is an indicator of the excellent stability of colloidal dispersions. Similar results have also been reported for the NC isolated from different plant species [39].

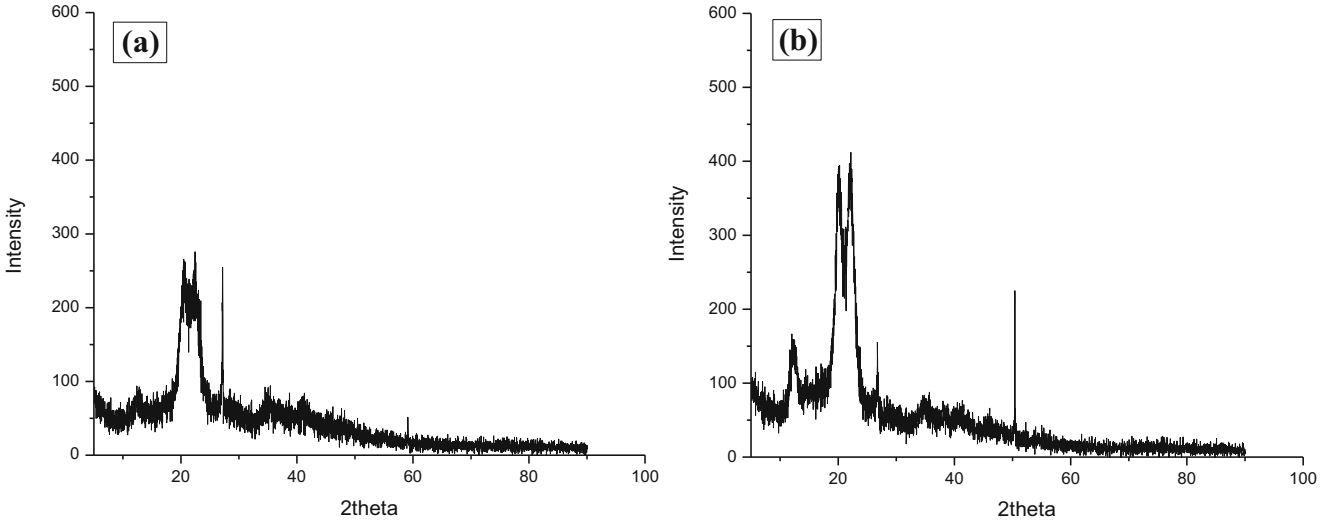

Fig. 5 X-ray diffractogram of **a** α-cellulose (αUSSP02) and **b** α-NC (αUSSPNC02)

6.4.6 Powder X-ray diffraction analysis

Cellulose exists in both amorphous and crystalline forms. Powder X-ray diffraction (XRD) analysis indicates the amount of crystalline form of cellulose. The crystallinity index of the both α-cellulose (αUSSP02) and α-NC (αUSSPNC02) was found to be 47.58% and 53.30%. The XRD data has shown a significant increase in the crystallinity of cellulose after the acidic hydrolysis which is mainly due to removal of amorphous regions under acidic conditions (Fig. 5). The crystalline index of cellulose obtained from cow dung represents similar values with the other plant and marine biomass [40, 41]. High crystallinity is required in preparation of composites.

7 Conclusion

The current study concluded that cow dung can act as an ideal feedstock for the extraction of cellulose, hemicellulose, and lignin. Cow dung biomass has been utilised for the extraction of cellulose and preparation of nanocellulose. The extraction of cellulose was carried out using the optimised kraft pulping process with minimal possible chemical load. Although cellulose was isolated in moderate yield as compared to the plant biomass, it can be considered good feedstock for the development of several value-added products. Also, α-NC prepared from the extracted cellulose was found to have excellent surface charge which imparts good particle stability in dispersions. Thus, valorisation of cow dung provides an alternative for efficient utilisation of this waste biomass in an innovative manner. This alternative way of animal waste utilisation serves dual function; it helps in management of huge amount of animal waste and also helps in the development of useful biomaterials, thus represents a good example of "Waste to Wealth".

Authors' Contributions Kumar S. conceived the idea for this work. Puri S. performed the experiments of cellulose pulp extraction and characterisation. Sharma S. performed the experiment on nanocellulose preparation. Sharma U., Sharma, M., and Kumari A. designed and coordinated the study. Manuscript is drafted and finalised by Sharma U. Sharma, M., Kumari A., and Kumar, S. All authors have read and approved the final manuscript.

Funding This research work is supported by CSIR-Agri Nutri Mission (MLP-0141). CSIR-IHBT Communication No. for this manuscript is 4647.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Statista (2020) Number of cattle worldwide from 2012 to 2020. https://www.statista.com/statistics/263979/global-cattle-population-since-1990/. Accessed 21 Oct 2020
2. Soyer G, Yilmaz E (2020) Waste management in dairy cattle farms in Aydin Region. Potential of energy application. Sustainability 12(4):1614
3. Yeck RG (1981) Managing dairy wastes. J Dairy Sci 64(6):1358–1364
4. Dhama K, Chauhan RS, Singhal L (2005) Anti-cancer activity of cow urine: current status and future directions. Int J Cow Sci 1(2): 1–25
5. Kleinman PJA, Spiegal S, Liu J, et al (2020) Managing animal manure to minimize phosphorus losses from land to water. In: Animal manure: production, characteristics, environmental concerns, and management 67, 201–228. Published by American Society of Agronomy, Inc. Soil Science Society of America, Inc, 5585 Guilford Rd., Madison, WI 53711, USA
6. Kaur G, Brar YS, Kothari DP (2017) Potential of livestock generated biomass: untapped energy source in India. Energies 10(7):847
7. Christov LP, Akhtar M, Prior BA (1998) The potential of biosulfite pulping in dissolving pulp production. Enzym Microb Technol 23(1–2):70–74

8. Seale RD, Sellers T Jr, Fuller MJ (1996) Kenaf core board material. U.S. Patent No. 5492756, Washington, DC
9. Jústiz-Smith NG, Virgo GJ, Buchanan VE (2008) Potential of Jamaican banana, coconut coir and bagasse fibres as composite materials. Mater Charact 59(9):1273–1278
10. Dhugga KS (2007) Maize biomass yield and composition for biofuels. Crop Sci 47(6):2211–2227
11. Pereira SC, Maehara L, Machado CMM, Farinas CS (2015) 2G ethanol from the whole sugarcane lignocellulosic biomass. Biotechnol Biofuels 8(1):44
12. American Chemical Society (2018) Elephant and cow manure for making paper sustainably. Press releases presented at a meeting of the American Chemical Society, held in New Orleans, March 21, 2018
13. Zulkifli ZB, Rasit NB, Umor NA, Ismail S (2018) The effect of A. Fumigatus SK1 and trichoderma sp. on the biogas production from cow manure. Malaysian J Fundam Appl Sci 14(3):353–359
14. Brinchi L, Cotana F, Fortunati E, Kenny JM (2013) Production of nanocrystalline cellulose from lignocellulosic biomass: technology and applications. Carbohydr Polym 94(1):154–169
15. Abitbol T, Rivkin A, Cao Y, Nevo Y, Abraham E, Ben-Shalom T, Lapidot S, Shoseyov O (2016) Nanocellulose, a tiny fiber with huge applications. Curr Opin Biotechnol 39:76–88
16. Saba N, Jawaid M (2017) Recent advances in nanocellulose-based polymer nanocomposites. In: Cellulose-reinforced nanofibre composites. Elsevier, pp. 89–112. Woodhead Publishing Series in Composites Science and Engineering. Universiti Putra Malaysia, Selangor, Malaysia
17. Khalil HPSA, Davoudpour Y, Islam MN et al (2014) Production and modification of nanofibrillated cellulose using various mechanical processes: a review. Carbohydr Polym 99:649–665
18. Nasir M, Hashim R, Sulaiman O, Asim M (2017) Nanocellulose: preparation methods and applications. In: Cellulose-reinforced nanofibre composites. Elsevier, pp. 261–276. Woodhead Publishing Series in Composites Science and Engineering, Malaysia
19. Deepa B, Abraham E, Cordeiro N, Mozetic M, Mathew AP, Oksman K, Faria M, Thomas S, Pothan LA (2015) Utilization of various lignocellulosic biomass for the production of nanocellulose: a comparative study. Cellulose 22(2):1075–1090
20. Runge T, Houtman C, Heinricher J, Negri A (2013) Timber bamboo pulp. TAPPI J 12(2):9–15
21. Kim D, Cheon J, Kim J et al (2017) Extraction and characterization of lignin from black liquor and preparation of biomass-based activated carbon therefrom. Carbon Lett (Carbon Lett) 22:81–88
22. Tappi T (2002) 222 om-02: acid-insoluble lignin in wood and pulp. 2002–2003 TAPPI test methods
23. Evans R, Wallis AFA (1989) Cellulose molecular weights determined by viscometry. J Appl Polym Sci 37(8):2331–2340
24. Burrell H, Immergut B (1975) Polymer handbook, 2nd edn. John Wiley, New York IV-337
25. Johnston HK, Sourirajan S (1973) Viscosity–temperature relationships for cellulose acetate–acetone solutions. J Appl Polym Sci 17(12):3717–3726
26. Tappi T (1993) Ash in wood, pulp, paper and paperboard: combustion at 525 C. TAPPI test methods T 211:67–64
27. Park S, Baker JO, Himmel ME, Parilla PA, Johnson DK (2010) Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biotechnol Biofuels 3(1):10
28. Conca RJ, Hamilton JK, Kircher WH (1963) Haze in cellulose acetate. Tappi 46(11):644
29. Malm CJ, Tanghe LJ (1963) Factors during the making of cellulose acetate which influence false viscosity. Tappi 46(10):629
30. Batalha LAR, Colodette JL, Gomide JL et al (2012) Dissolving pulp production from bamboo. BioResources 7(1):640–651
31. Hemmasi AH, Samariha A, Tabei A et al (2011) Study of morphological and chemical composition of fibers from Iranian sugarcane bagasse. Am J Agric Environ Sci 11(4):478–481
32. Fengel D, Shao X (1984) A chemical and ultrastructural study of the bamboo species Phyllostachys makinoi Hay. Wood Sci Technol 18(2):103–112. Chapter -5 and Elsevier Inc
33. Ge X, Chang C, Zhang L, et al (2018) Conversion of lignocellulosic biomass into platform chemicals for biobased polyurethane application. In: Advances in bioenergy, vol. 3. 161–213
34. Toğrul H, Arslan N (2003) Flow properties of sugar beet pulp cellulose and intrinsic viscosity–molecular weight relationship. Carbohydr Polym 54(1):63–71. American Chemical Society: Washington, DC
35. Sun R, Sun XF, Tomkinson J (2004) Hemicelluloses and their derivatives. In: ACS Symposium SeriesVol. 864. ACS Publications, pp 2–22
36. Pastorova I, Botto RE, Arisz PW, Boon JJ (1994) Cellulose char structure: a combined analytical Py-GC-MS, FTIR, and NMR study. Carbohydr Res 262(1):27–47
37. Pappas C, Tarantilis PA, Daliani I, Mavromoustakos T, Polissiou M (2002) Comparison of classical and ultrasound-assisted isolation procedures of cellulose from kenaf (Hibiscus cannabinus L.) and eucalyptus (Eucalyptus rodustrus Sm.). Ultrason Sonochem 9(1): 19–23
38. Morais JPS, Rosa MDF, De Souza FMDSM et al (2013) Extraction and characterization of nanocellulose structures from raw cotton linter. Carbohydr Polym 91(1):229–235
39. Singla R, Soni S, Kulurkar PM, Kumari A, S. M, Patial V, Padwad YS, Yadav SK (2017) In situ functionalized nanobiocomposites dressings of bamboo nanocellulose and silver nanoparticles for accelerated wound healing. Carbohydr Polym 155:152–162
40. Rambabu N, Panthapulakkal S, Sain M, Dalai AK (2016) Production of nanocellulose fibers from pinecone biomass: evaluation and optimization of chemical and mechanical treatment conditions on mechanical properties of nanocellulose films. Ind Crop Prod 83:746–754
41. Bettaieb F, Khiari R, Hassan ML, Belgacem MN, Bras J, Dufresne A, Mhenni MF (2015) Preparation and characterization of new nanocellulose from marine biomass Posidonia oceanica. Ind Crop Prod 72:175–182