

Microcrystalline cellulose powder from banana pseudostem fibres using bio-chemical route

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Microcrystalline cellulose powder was prepared from banana pseudostem fibres. The conventional method for preparing cellulose powder requires cooking of raw material with high concentration of alkali followed by washing, bleaching and grinding. In this study cellulose powder was prepared by a method which was developed by CIRCOT in which raw material (banana fibres) were subjected to anaerobic treatment in the presence of microbial consortium for 7 days at room temperature. Fibres were washed with water and cooked in 1 % alkali at 100° C for 30 min. followed by bleaching and thorough washing. Hydrolysis of bleached pulp was carried out using 2.5N HCl to get the microcrystalline cellulose powder and studied the properties of the produced cellulose powder such as moisture content, ash content, cellulose content, starch content, pH value, solubility, degree of polymerization, molecular weight, particle size, degree of compressibility, x-ray crystallinity and infrared spectroscopy. Microcrystalline cellulose (MCC) powder prepared from banana fibres has properties at par with commercial grade MCC.

Keywords: Pseudostem, Banana fibres, Anaerobic treatment, Microcrystalline cellulose.

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Introduction

During the past decade, there has been a dramatic increase in cellulose research due to significant advances in cellulosic modifications (mechanical, chemical and enzymatic) and subsequent development of products with unique chemical and physical properties. This renewed focus on cellulose and its derivatives is based on several factors, such as the increased awareness of the availability of cellulose sources, for example agricultural waste products, the development of new solvent systems for cellulose activation/solubilization and the development of region-selective modification methodologies^{1,2}.

Cellulose is used widely as a raw material for a number of excipients used in the pharmaceutical industry. Microcrystalline cellulose (MCC), a purified, partially depolymerised, non-fibrous form of cellulose, is perhaps the best filler-binder currently available. MCC is available from different manufacturers having different trade names. MCC is prepared by hydrolyzing native α -cellulose, a fibrous,

semi-crystalline material, with dilute mineral acids. During hydrolysis, the accessible amorphous regions are hydrolyzed and a lower degree of polymerization product is obtained. Generally, it is understood that the differences in the properties of MCC between different manufacturers are due to the type of pulp used as raw material and processing conditions. Since cellulose from different sources have different properties it is expected that MCC obtained from different sources would also have different properties. These differences can affect their functionality when used as excipients. Excipients are generally chosen based on their compatibility and functionality to ensure the stability and bioavailability of the finished drug, as well as, the potential for large scale manufacturing. The chemical composition and physical structure of MCC depends significantly on the characteristics of the raw material used and the processing conditions³.

MCC works as an excellent filler/binder and exhibits a high dilution potential⁴. Preparation of MCC from materials other than wood pulp and cottons linters such as reed stalks, wheat and rice straws, jute, water hyacinth, sugarcane bagasse, coconut shells, Indian bamboo, soybean husk, luffa fibers, flax straws, cotton stalks, groundnut husk and

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orange mesocarp have been reported⁵⁻²⁰. There are many solutions available to overcome the shortage of MCC like recycling of paper and its products. The usage of fibres from fast growing and high biomass yielding non woody plants, are to be considered as the alternative source of raw materials²¹. Banana is one which yields high biomass and it may serve as an alternative raw material to fibre based industries like paper, card board, tea bags, fibre lining for car interiors, high quality dress material and currency notes²².

Banana is the common name for herbaceous plants of the genus *Musa* L. family *Musaceae*²³. The production of banana in India was about 27.01 million tonnes from an area of 0.765 million ha, ranking number one in the world production in 2011^(Ref.24). Research work done on the effective utilization of the huge biomass generated after the harvest of the fruit bunches i.e. the pseudostem is limited. At present, the pseudostem is dumped on road side or burnt or left *in situ*. As a result, this contributes to the greenhouse effect, soil erosion and pollution of the atmosphere and water sources. However, it is has been exploited for various uses like fibre, fertilizer and paper making. The preparation of MCC from banana stem and rice straw was carried out using alkali hydrolysis process followed by further treatment with peracetic acid²⁵. Banana stems MCC prepared by three steps delignification, bleaching and acid hydrolysis has been studied earlier²⁶.

In this paper, an attempt has been made to standardize a bio-chemical route for preparation of MCC from banana pseudostem fibres. The prepared MCC was studied for various physical and chemical characteristics. The conventional process for preparation of microcrystalline cellulose powder requires large quantity of chemicals and energy, whereas, the CIRCOT process is highly energy saving, low cost investment, environmentally safe and of low chemical requirement due to adoption of microbial route.

Materials and Methods

Banana fibres, a ligno-cellulosic fibre, obtained from the stem of banana plant, is a bast fibre with relatively good mechanical properties. In order to determine the chemical composition of banana pseudostem fibres, five popular varieties namely, 'Grand Naine', 'Poovan', 'Adhar Velchi', 'Sakar Chayna' and 'Vannan' were selected. The fibres

were cut into small pieces of about 1.5 to 2.0 cm and then pulverised in a laboratory pulveriser to a mesh size of about 50 μm . The pulverised material was used for determining the chemical constituents. The determination of ash (TAPPI T-211), estimation of Hot Water Solubles (T207 OM-81-1981), estimation of Alcohol-Benzene Extractable Matter (TAPPI T204 OS-76-1976), estimation of Lignin (T222 OS-74-1974) and estimation of Holocellulose (TAPPI T-9, M-54) was carried out using TAPPI standards²⁷ and the results are presented in Table 1.

'Grand Naine', most popular variety of banana at central India region, was selected for further experimental purpose. Fibres were mechanically extracted using raspador to remove pithy matter and then further washed and dried in sun light. All the chemicals used were of analytical reagent grade.

Dry banana fibres weighing 500 g were cut into pieces of 2-3 cm and were soaked in 5 % (v/v) nitric acid at room temperature for 24 h keeping material to liquor ratio at 1:10. The fibres were washed thoroughly with water and subjected to anaerobic treatment for 7 days. This anaerobic treatment process was earlier standardized²⁸ for the preparation of binderless boards from cotton plant stalk by giving treatment for 1 day to 7 days. The results indicated that there was not much change in the chemical composition after one day of anaerobic treatment also better results were obtained with 7 days of treatment. The fibres were transferred to ten litre capacity bottles containing stabilized microbial consortium. The bottles were stoppered with rubber corks and sealed with paraffin wax. From the side arms, rubber tubes were connected as air traps to prevent the entry of air. After the incubation period, the sample was taken out and

Table 1—Chemical composition of banana pseudostem fibres (Dry weight basis)

Variety Name	Ash %	Hot water soluble %	Alcohol: benzene %	Lignin %	Holo-cellulose %
'Grand Naine'	6.0	8.1	2.6	9.1	74.2
'Poovan'	4.8	6.2	1.3	11.0	76.7
'Adhar Velchi'	3.1	10.4	2.0	8.8	75.7
'Sakar Chayna'	4.4	9.3	1.9	8.9	75.5
'Vannan'	3.1	7.4	0.9	10.3	78.3

washed with water followed by open boiling with 1 % (w/w) sodium hydroxide for 15 min. The fibres were again washed with water, neutralized with 0.2 % (v/v) acetic acid and bleached. The bleaching was carried out using hydrogen peroxide (0.3 % w/v), along with sodium hydroxide (0.1 % w/v) and sodium silicate (0.15 % w/v) as stabilizer at 90-95° C for 30 min with material to liquor ratio 1: 20. The bleached sample was then subjected to acid hydrolysis using 2.5 N hydrochloric acid. The hydrolyzed material was filtered, washed free of acid, dried and powdered using pulverizer of 0.053 mm mesh size. The obtained product i.e. MCC was studied for following various physical and chemical parameters.

Moisture content

A sample weighing of 2 g (A) was taken in tared weighing bottle. It was dried in oven for two hours at $105^{\circ} \pm 3^{\circ}$ C and then cooled in desiccator. The stopper was momentarily opened to equalize the air pressure and weighed again. Once again the bottle was returned to oven for one hour, cooling and weighing and this action was repeated for successive hourly periods until a constant weight (B) was obtained. The Moisture Content (%) was calculated using the formula:

$$\text{Moisture content} = ((A-B)/A) \times 100$$

Ash content

The ash content was determined by heating a sample in a muffle furnace²⁷. 5 g of sample was placed in crucible and weighed. The sample then ignited over the Bunsen Flame until it was carbonized. The crucible was then transferred to a muffle furnace and the ashing continued at 750°C, until it attains a constant weight. The ash content was expressed as a percentage of oven dry weight of the material.

Cellulose content

Cellulose content in MCC powders was estimated as per the method of Halliwell²⁹. About 2 g of the samples were extracted with ether for 6 h in a Soxhlet apparatus. Defatted samples were boiled in 0.5 % ammonium oxalate (1: 50) for 1 h to remove water soluble and pectin. Subsequently, they were bleached using 1 % sodium chlorite solution in 0.05 N acetic acid (1: 30) in a boiling water bath for removing lignins. The resultant holocellulose

was treated first with 5 % potassium hydroxide followed by 24 % potassium hydroxide for 2-3 h. The cellulose thus obtained was stirred with a 20 fold volume of concentrated phosphoric acid for 2 h at 1°C, washed with 1 % solution of sodium carbonate and then with water. The residue was dried and weighed to constant weight and reported as percent cellulose. The percentage of ash was determined and the weight of cellulose was corrected by subtracting the weight of ash.

Starch content

Cellulose suspension was prepared by taking 2 g sample in 100 mL distilled water (USP, 1980). Few drops of iodine solution of 1 % was added to 20 mL of suspension and mixed thoroughly. The development of blue colour showed the presence of starch³⁰.

Determination of pH

1 g of sample of cellulose powder was weighed accurately and suspended in about 500 mL distilled water. The suspension was allowed to stand for half an hour. Then the pH of 2 % suspension was determined using Beckmann pH Meter³⁰.

Solubility characteristics

Solubility in water was determined by shaking 4.0 g of sample with 80 ml of water for 10 minutes, filtered through Whatman filter paper into a preweighed beaker, evaporated on a steam bath to dryness at 105°C for 1 h³¹. Solubility in 1 % alkali was determined by heating 2.0 g of sample with 1% sodium hydroxide (100 mL) on a water bath. After 1 h insoluble residue in the beaker was filtered through glass crucible, washed with 1.0 % acetic acid and finally with hot water, dried to constant weight. Loss in weight of the dry sample was calculated as material soluble in 1 % sodium hydroxide.

Solubility in 1 % acid (hydrochloric acid) was determined by shaking 2.0 g of sample with 100 mL of 1 % hydrochloric acid for 1 h, filtered through glass crucible and the residue was washed with 0.1N sodium hydroxide and finally with hot water. It was dried to constant weight, the loss in weight of the sample was calculated as the material soluble in 1 % acid.

Solubility in organic solvent was determined by extracting 2.0 g of sample with distilled petroleum ether (Boiling point 40 - 60°C) for 5 h in Soxhlet

extractor. After that extracted sample was taken in the beaker, solvent was evaporated by heating on water bath and dried at 105°C to constant weight.

Solubility in oil was determined by shaking the known quantity of sample that is 2.0 g with oil (groundnut, 50 mL) for 4 h. After 4 h insoluble residue in the flask was filtered through glass crucible, washed the residue with water and dried to constant weight. The loss in weight of the sample was calculated as material soluble in oil.

Degree of polymerization

Molecular size is expressed as the number of anhydro glucose units in a chain (the degree of polymerization). The degree of polymerization (DP) was carried out as per the standard method³² using 0.5 % solution of the sample in cuprammonium hydroxide using Shirley type Viscometer. The DP was calculated from the following formula.

$$DP = 2160 [\log (nr+1) - 0.267]$$

where nr = Relative Viscosity

Molecular weight was calculated from the degree of polymerization by multiplying the value of DP with the molecular weight of glucose i.e. 162.

Determination of particle size

Particle size of both MCC powder samples were analysed using Scanning Electron Microscope. MCC sample was sprinkled on a SEM Stub fixed with double sided adhesive tape. The sample was coated with gold palladium in sputter coater to avoid charging and examined in Stereoscan SEM S-150 at 10 KV with the tilt angle of 45°. Using suitable magnification, micrographs were recorded. Typical SEM micrographs of MCC were selected and from the SEM image approximate size of the MCC was determined.

Degree of compressibility

Bulk density is defined as the mass of powder divided by the bulk volume³³. Cellulose powder was passed through a standard sieve of 80 µm mesh size and introduced into a 25 mL capacity of graduated glass cylinder. At this stage aerated bulk density (A) was obtained by the mass of the sample observed in the cylinder. Further, same cylinder was tapped on a hard wooden surface 15 times from height of one inch, at two seconds intervals. The packed bulk density (P) was then obtained by dividing the weight of the sample in grams by the final volume

of the sample observed in the cylinder. Then % compressibility was calculated by using following expression:

$$\% \text{ Compressibility} = [(P-A) / A] \times 100$$

Where P= Packed Bulk Density and A = Aerated Bulk Density

X-Ray crystallinity

To calculate X-Ray crystallinity, the method followed by Ferrow and Pretson³⁴ was used. The degree of crystallinity was determined using an X-ray powder diffractometer (PANalytical X'Pert PRO X-Ray Diffractometer). The samples for analysis were prepared by pressing the powder into the cavity of a sample holder and smoothing with a glass slide. They were scanned from 5-40° 2θ with a Cu anode X-ray operated at 40 kV and 40 mA in combination with a Ni filter to give a monochromatic Cu-K radiation ($\lambda = 1.5418 \text{ \AA}$). The Crystallinity Indices (CI) of the specimens were calculated by the following equation as proposed by Segal's *et al*³⁵.

$$CI = 100 \times \frac{I_{002} - I_{am}}{I_{002}}$$

where I_{002} is the maximum intensity of the principal peak (002) lattice diffraction (at 22.7° of 2θ for cellulose I and 21.7° of 2θ for cellulose II), and I_{am} is the intensity of diffraction attributed to amorphous cellulose. Integration of the crystalline reflection was carried out using OriginPro Software.

Infrared spectroscopy

Fourier transform infrared (FT-IR) spectra were recorded using KBr discs on a PerkinElmer FT-IR spectrometer. KBr pellets containing 1% of the samples were prepared. The scanned range was 4550-400/cm with a resolution of 4/cm and 32/cm scans per sample. For the determination of crystallinity in the cellulosic material, the ratio A_{1372} / A_{2900} was considered, where A_{1372} and A_{2900} are the areas of the peaks at 1372/cm and 2900/cm, respectively³⁶. Integration of the peak areas was carried out using OriginPro software.

Results and Discussion

Chemical composition of banana pseudostem fibres are presented in Table 1. It is found that banana fibres have holocellulose in the range of 74.2 to 78.3%. Comparison of various physico-chemical properties of cellulose powder prepared from banana pseudostem with commercial MCC are presented in Table 2.

Table 2—Comparison of various physico-chemical properties of MCC prepared from banana pseudostem fibres

S. No.	Property	MCC prepared from banana fibres	Commercial MCC	Standards as per USP/IP
1.	Moisture content (%)	5.3	5.0	< 10
2.	Ash content (%)	0.07	0.06	< 0.1
3.	Cellulose content (%)	99.0	100	≥ 97
4.	Starch	0	0	0
5.	pH value	6.4	7	5.5-7
6.	Solubility			
	Distilled water	Partially soluble	Partially soluble	Partially soluble
	1% NaOH	Partially soluble	Partially soluble	Partially soluble
	1% HCL	Insoluble	Insoluble	Insoluble
	Petroleum ether	Completely insoluble	Completely insoluble	Completely insoluble
	Acetone	Completely insoluble	Completely insoluble	Completely insoluble
	Ground nut oil	Completely insoluble	Completely insoluble	Completely insoluble
7.	Degree of polymerization	267	157	-
8.	Molecular weight	43254	25434	-
9.	Particle size in μm	30-60	20-100	10-50
10.	Degree of compressibility (%)	24.9	11.0	-
11.	X-ray crystallinity (%)	72.1	82.1	-

Moisture content of banana fibre MCC is 5.3 % which is 0.3% more than that of commercial MCC. According to U.S. Pharmacopoeia 1980, the possible effect of moisture is expected to be negligible for the moisture content below 10 %. The ash content of cellulose powder from banana fibre was 0.07 % which is in close agreement with ash content (0.06 %) of commercial sample and according to U.S. Pharmacopoeia 1980, ash content should not be more than 0.1 %. The cellulose content in MCC prepared from banana fibres is 99.0 % which is more than the standard. According to U.S. Pharmacopoeia³⁰ cellulose content should not be less than 97 %. Hence, this product stands well in agreement with U.S.P. specifications. The starch content in cellulose powder prepared from banana fibre is nil which is at par with MCC in U.S.P. The pH value of prepared MCC is 0.6 less

than that of commercial material. Prepared cellulose powder as well as commercial MCC is having similar characteristics for solubility in water, dilute alkali, dilute acid, organic solvent and groundnut oil which is as per standards of Pharmacopoeia of India³⁷.

Particle size of the prepared MCC is in narrow range of 30 μm whereas commercial MCC is having 80 μm range. Sample of SEM Micrographs for banana MCC and commercial MCC are presented in Fig.1 to Fig. 4, respectively. It can be observed from the SEM micrographs that banana MCC surface is plain without any serrations (Fig. 3 and Fig. 4 for banana MCC and Commercial MCC, respectively). Particle size depends upon the extent of degradation of cellulose molecule. The cellulose powder prepared from banana fibres have lower molecular degradation than commercial MCC.



Fig. 1—SEM Micrographs of Powdered MCC prepared from banana pseudostem



Fig. 2—SEM Micrographs of Commercial MCC powder



Fig. 3—SEM Micrographs of Powdered MCC prepared from banana pseudostem (surface topography)

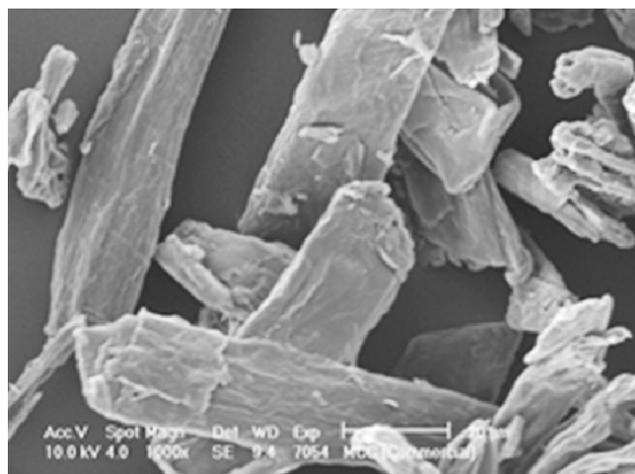


Fig. 4—SEM Micrographs of Commercial MCC powder (surface topography)

The cellulose powder prepared from banana fibres is found to have 13.9 % more compressibility than commercial MCC.

Powder X-ray diffraction spectra of the two cellulose samples are shown in Fig. 5 which showed typical of Cellulose I structure with diffraction peaks of the 2 angles at 15° , 16.5° and 22.6° . The sharp peak of 22.6° indicates higher crystallinity. Percentage crystallinity of cellulose powder prepared from banana fibres is 72.1 % whereas for commercial MCC is 82.1 %.

The Crystallinity Index gives a quantitative measure of the crystallinity in powders which can be related to the strength and stiffness of fibres³⁸. High crystallinity indicates an ordered compact molecular structure which translates to dense particles whereas lower crystallinity implies a more disordered structure, resulting in a more amorphous powder. As seen in Fig. 6 of FTIR spectra, MCC from banana fibres showed the similar characteristic vibration peaks of cellulose^{39,40}.

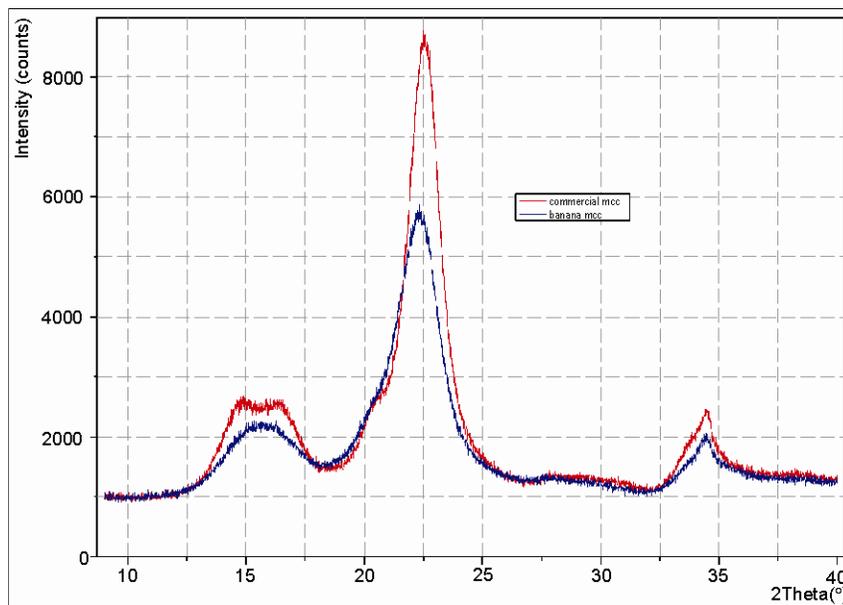


Fig. 5—X-ray diffractograms of the cellulose samples

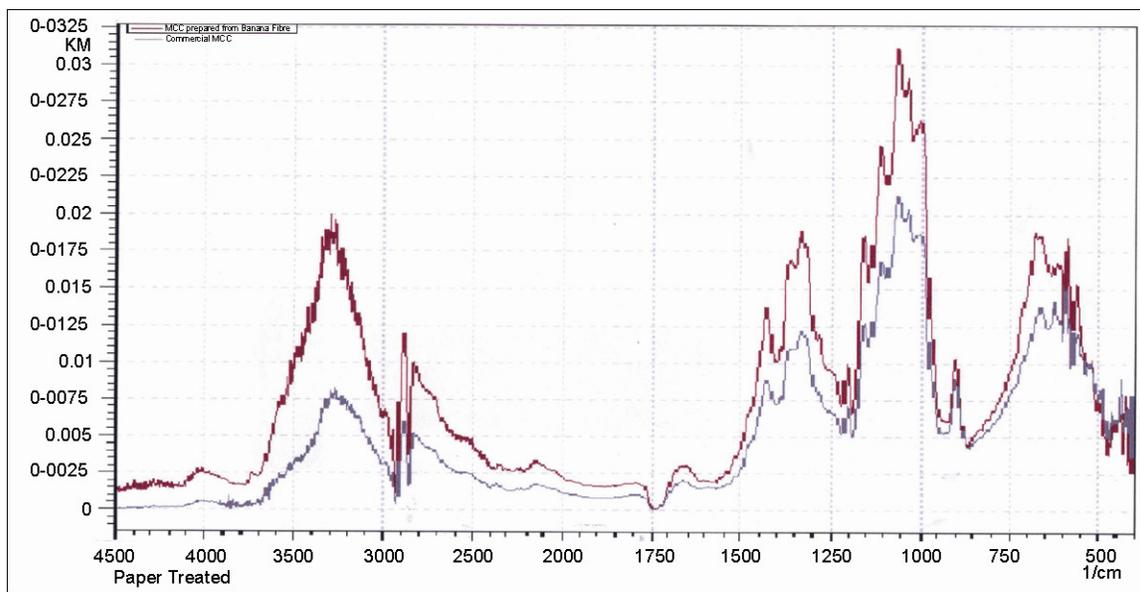


Fig. 6—Comparison of FTIR spectra of MCC produced from banana fibres with commercial MCC

Conclusion

Banana fibres are rich in cellulose and thus can be a potential raw material for the preparation of microcrystalline cellulose. The MCC prepared from banana fibres meets the U.S.P. specifications and has physical and spectroscopic properties at par with the commercial grade of microcrystalline cellulose. The results also confirm that one can

use a biochemical route for preparation of MCC. Biochemical route is environmentally safe and minimizes the use of chemicals.

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