

Chapter 3: Materials and methods

The banana trunk used in the present work was powdered (explained in the next section) and subjected to acid and alkali treatment. Characterization of both treated and untreated biomass powder was done to determine its cellulose, hemi-cellulose, lignin and ash contents. The films prepared using various casting solutions were characterized for determining their tensile strength, elongation at break-point, contact angle, and water vapor permeability.

Poly vinyl alcohol, MW ~1,40,000, 95% hydrolyzed was purchased from Himedia, Citric Acid, Sodium Hydroxide, Sulphuric acid, Maleic acid, glycerol, sorbitol, fructose, glutaraldehyde etc were purchased from Merck. Water wherever used was double distilled water prepared in lab using Borosil double distillation plant.

3.1 Extraction of lignocellulosic fibres from banana pseudostem:

The banana pseudostem was obtained from local agricultural field near IIT BHU Varanasi. Banana fibers were extracted as follows: the pseudostem was cut and layers removed manually. After washing thoroughly with water the pseudostem layers were subsequently sun dried for 6 - 8 days then dried in oven for 24 hours at 80° C to remove residual moisture. The dried layers were subsequently cut into pieces of 10 x 5 cm and powdered in pulverizer by Wiley mill (Model 2, Arthur H. Thomas Co. Philadelphia, USA) followed by sieving by sieves of mesh no. 170, no. 200 and no. 270 to collect banana pseudostem fibers of size range <53 um, 53-75 um and 75 – 90 um. These fibers were then used either in treated or untreated condition.



Fig. 3.1(a) Layers of banana pseudostem; (b) Layers after drying; (c) Pulverised and sieved BPF

3.2 Compositional analysis of lignocellulosic fibers

3.2.1 Lignin content (ASTM D 1106-96):

Two grams of sample were placed in a flask and 15 ml of 72% sulphuric acid was added. The mixture was stirred frequently for two and a half hours at 25°C and 200 ml of distilled water were added to the mixture. The mixture was boiled for two hours and cooled. After 24 hours the lignin was transferred to crucible and washed with hot water repeatedly until acid free. The collected lignin was dried at 105°C and cooled down in dessicator and weighed. The cycle was repeated until constant weight.

3.2.2 Holocellulose content (ASTM D 1104-56):

Three grams of dried samples were weighed and placed in an Erlenmeyer flask and 160 ml distilled water; 0.5 ml glacial acetic acid and 1.5 g of sodium chloride were added.

The flask was placed in water bath at 75°C for an hour and then additional 0.5 ml glacial acetic acid and 1.5 g sodium chloride were added. The additions of acetic acid and sodium chloride were repeated two times hourly. The flask was placed in an ice bath and cooled down below 10°C. The holocellulose was filtered and washed with acetone, ethanol and water respectively and at the end sample was dried at 105°C and weighed.

3.2.3 α -cellulose content (ASTM D 1103-60):

Two grams of holocellulose were placed in a beaker and 10 ml of 17.5% sodium hydroxide was added. The fibres were stirred up by glass rod. Sodium hydroxide solution were added to the mixture periodically (once every 5 minutes) for half an hour and the mixture temperature was kept at 20°C. 33 ml of distilled water was added in the beaker and kept for 1 hour. The holocellulose residue was filtered and transferred to the crucible and washed with 100 ml of sodium hydroxide (8.3%), 200 ml of distilled water, 15 ml acetic acid (10%), and again water successively. The crucible with α -cellulose was dried and weighed. Hemicellulose content was calculated as

$$\text{Hemicellulose} = \text{Holocellulose} - \alpha\text{-cellulose}$$

3.2.4 Ash content (ASTM D 1102-84):

Silica crucible was preheated at 580°C for 4 hours in a muffle furnace and then placed in dessicator and allowed to cool down for 2 hours. The crucible was weighed and 1g of BPF pre-dried in hot air oven at 105°C for 4 hours were placed in crucible and kept in pre-heated muffle furnace set at 575°C for 24 hours. After 24 hours the crucible with ash was weighed again and ash content was calculated as follows:

$$\text{Ash content} = \text{wt of crucible with ash} - \text{wt of crucible.}$$

3.3 Pretreatment of fibers

3.3.1 Alkali treatment of Banana Pseudostem Fibres:

20 g of Banana pseudostem fibres (BPF) were soaked in 300 ml of Sodium hydroxide solution of varying strength (1%, 2.5%, 5%, 7.5%, 10% w/v) in a water bath for 1 hour at 90° C with continuous stirring. The fibres were then washed in a vacuum filtration setup with distilled water until neutral pH.

3.3.2 Acid Treatment of Banana Pseudostem Fibres:

20 g of Banana pseudostem fibres (BPF) were soaked in 300 ml of Sulphuric acid solution of varying strength (1%, 2.5%, 5%, 7.5%, 10% w/v) in a water bath for 1 hour at 90° C with continuous stirring. The fibres were then washed in a vacuum filtration setup with distilled water until neutral pH.

3.4 Green synthesis of nanocellulose from microcrystalline cellulose (Adsul *et al.*, 2012)

Alkali – urea solution and ultrasonication was used for breaking the chains of microcrystalline cellulose (MCC) to produce nanocellulose. 5 g MCC was added to a solution containing NaOH 7% (w/w) and urea 12% (w/w). The mixture was stirred for 30 minutes at 25°C and later refrigerated at -20°C for 16 – 20 hrs. After refrigeration the mixture was vigorously stirred until complete dissolution of MCC. 1 L double distilled water was added to regenerate the dissolved MCC. The mixture was centrifuged at 3000 rpm for 10 minutes followed by washing with double distilled water to a pH range of 7 - 9. The solution was then ultrasonicated for 5 minutes to obtain suspension of dispersed nanocellulose which was stored at 4°C until use.

3.5 Characterization of synthesized nanocellulose

The prepared nanocellulose was characterized by FTIR spectroscopy and X-Ray diffraction analysis. For estimation of particle size diameter Dynamic Light Scattering (DLS) analysis was done using a Particulate Systems NanoPlus-3, Micromeritics, Norcross, U.S.A. FTIR and XRD analysis of prepared nanocellulose was carried out as detailed in Section 3.8.2 and 3.8.3 respectively.

3.6 Citric acid modification of Sodium – Montmorillonite clay

About 230 ml of distilled water was heated to 80°C in a glass beaker over a magnetic stirrer equipped with hot plate. 1.68 g of citric acid (about 8.75mmol) and 0.8 ml of sulphuric acid (98%) was added to the preheated water. A suspension of 5 g clay in 100 ml water was prepared by stirring the clay in water for about 10 minutes. The acidic solution of citric acid was added to clay suspension and mixture stirred for 3 hours while maintaining temperature at 80°C. After cooling the solution was filtered and cake washed with distilled water by centrifuging for 30 minutes. The clay obtained was dried at 60°C for 24 hours and ground to a fine powder.

3.7 Preparation of PVA composite films

3.7.1 Preparation of PVA- banana pseudostem fiber composite films

PVA-BPF composite films were prepared by solution casting method. Briefly, polyvinyl alcohol, BPF, plasticizer, and cross linkers were suspended in 100 ml of distilled water. The mixture was slowly heated to 80°C with constant stirring until a homogeneous solution was formed. Water was added to compensate for any evaporative loss that may have occurred during the heating process. The solution was ultrasonicated for 30 minutes to remove bubbles and then cast on glass casting plates of dimensions 150 mm x 150 mm x 4 mm. Film thickness was maintained by the volume of solution being cast

in the plates. The casting plates were incubated for 24 hours at room temperature and the film was peeled off from casting plates later. The films were used as such for characterization.

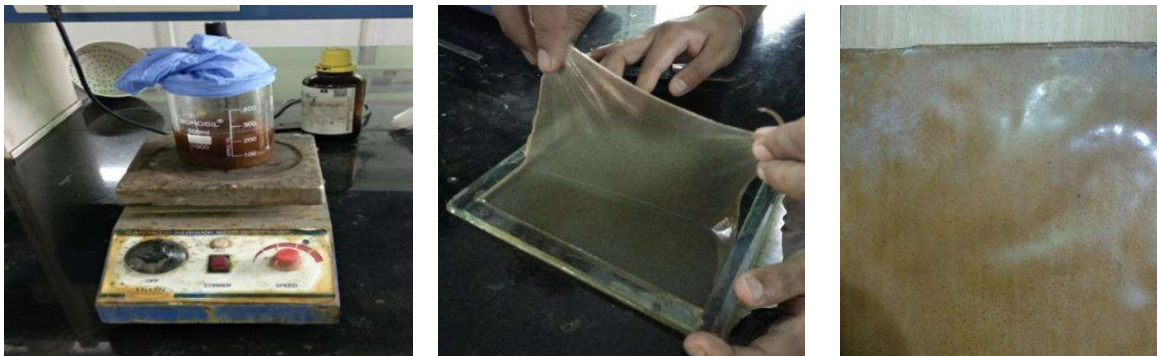


Fig. 3.2 (a) Preparation of film casting solution; (b) Peeling off of the dried film from casting plate; (c) PVA-BPF composite film

3.7.2 Preparation of PVA- banana pseudostem fiber (BPF) composite films reinforced with nanocellulose.

Composite films were prepared by solution casting method as previously reported (Srivastava *et al.*, 2019), with minor modifications. Briefly, polyvinyl alcohol 8g was dissolved in 100 ml distilled water by stirring for 40 minutes at 80°C. To this solution, 2 g BPF, plasticizer, citric acid and glutaraldehyde were added and stirred on magnetic stirrer for 15 minutes until homogenized mixture was obtained. Required amount of nanocellulose solution was then dispersed in the mixture and further mixed for another 40 minutes. Water was added to compensate for any evaporative loss that may have occurred during the mixing process. The solution was ultrasonicated for 10 minutes to remove air bubbles and then cast on glass casting plates of dimensions 150 mm x 150 mm x 4 mm. Film thickness was maintained by the volume of solution being cast in the plates. The casting plates were incubated for 24 hours at room temperature and the film was peeled off from casting plates later. The films were used as such for characterization.

3.7.3 Preparation of PVA- banana pseudostem fiber (BPF) composite films reinforced with nanoclay and citric acid modified nanoclay

The same method as that for nanocellulose reinforced film was used except that in place of nano cellulose required amount of nanoclay/ citric acid modified nanoclay aqueous suspension was dispersed in the mixture and further mixed for another 40 minutes. Water was added to compensate for any evaporative loss that may have occurred during the mixing process. The solution was ultrasonicated for 10 minutes to remove air bubbles and then cast on glass casting plates of dimensions 150 mm x 150 mm x 4 mm. Film thickness was maintained by the volume of solution being cast in the plates. The casting plates were incubated for 24 hours at room temperature and the film was peeled off from casting plates later. The films were used as such for characterization.

3.8 Characterization

3.8.1 Scanning Electron Microscopy (SEM):

The SEM-EDS of treated/ untreated fibers were recorded on ZEISS EVO 18 SEM coating of quorum Q150R ES having model number 51-ADD0048.

3.8.2 Fourier Transform Infrared spectroscopy (FT-IR):

FTIR analysis of both untreated and treated banana fibres and nano cellulose, nano clay and modified nanoclay was carried out with Thermo-Nicolet 5700 model. The Omnic software was used to correct the medium's background material used during analysis. Fourier transformed infrared spectrophotometer was set to a resolution of 4 cm^{-1} , in the range of $500\text{--}4000\text{ cm}^{-1}$ in transmission mode.

3.8.3 X-Ray Diffraction (XRD):

X-ray analysis of the treated and untreated fibres and nano cellulose, nano clay and modified nanoclay was carried out using Rigaku Ultima IVX-ray diffractometer

(Germany) for phase identification. The patterns were run with Cu-K α radiation at 40 kV and 40 mA. The XRD analysis was performed at 2°/ min.

3.8.4 Contact angle measurement of PVA-BPF composite films:

The prepared films were subjected to contact angle measurements for estimating the changes in hydrophilic behaviour of prepared composite films. This was done using sessile drop method on a KRUSS DSA25 Series, Germany Drop shape analysis system at room temperature. A droplet of water was dispensed on the film surface and allowed to interact with the film surface for 1 s. Immediately afterwards, the water contact angle of film was recorded. Average of two values recorded has been reported.

3.8.5 Water swelling test of PVA-BPF composite films:

Films were cut into squares of size 10 mm X 10 mm then weighed and placed in petri plates containing distilled water. The weight of films was taken every 24 hours after gently wiping them with tissue paper. This was repeated until nearly constant weight. Water swelling percent was calculated as

$$\text{water swelling (\%)} = \frac{wt-w_0}{w_0} \times 100 \%$$

where wt and w₀ are weights of films after time t and initially respectively. The experiments were performed in triplicates and mean along with standard deviation values have been reported.

3.8.6 Water vapour permeability of PVA-BPF composite films:

The desiccant method of ASTM method E – 96 (ASTM E 96 (1995), 1995) was used to determine the water vapour permeability. Poly methylmethacrylate cups were filled with previously dried anhydrous calcium chloride up to 1 cm from the film and then sealed with prepared composite films using wax. The cups were weighed and kept in a

desiccator containing saturated solution of potassium chloride at 25° C (RH: 85%).

Weight gained by the cups was recorded every 24 hours for a period of 10 days.

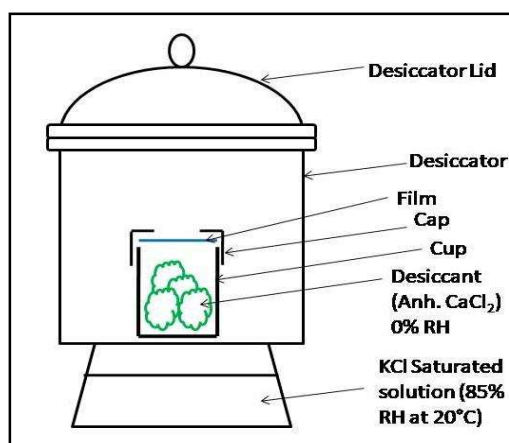


Fig. 3.3 Schematic of water vapour permeability setup

The weight gain per unit time was calculated from the linear portion of slope of the weight vs. time curve. This was divided by the area of the film exposed to get water vapour transmission rate, WVTR.

$$WVTR = \frac{\Delta w}{\Delta t} \frac{1}{A} \text{ g/day m}^2$$

Where Δw is the weight gain by the cups

Δt is the time duration in days

A is the area of film exposed.

Water vapour permeability was calculated as follows:

$$WVP = \frac{WVTR}{P^\circ (RH1 - RH2)} \cdot l \text{ g.m / s m}^2 \text{ Pa}$$

Where P° is the specific vapour pressure of saturated KCl solution at 25°C (2665 Pa) and RH1 and RH2 are the relative humidity's maintained inside the Desiccator (Vapour source, RH 85%) and the cups (Vapour sink, RH 0%) respectively. The experiments

were performed in triplicates and mean along with standard deviation values have been reported.

3.8.7 Tensile strength and elongation at break:

80 mm by 20 mm samples were cut from the prepared composite films and used for the tensile test using an Instron universal testing machine with a head speed of 2 mm per second. The mechanical testing was performed using ASTM D0882 having gauge length 25mm and film thickness of 120 micrometers. The experiments were performed in triplicates and mean along with standard deviation values have been reported.

3.8.8 Biodegradability of PVA-BPF composite films:

Film squares of 10 cm x 10 cm were cut and weighed. Weighed films were then buried 4 inches deep in moist garden soil in a pot kept at constant room temperature of about 30 °C. The pot was occasionally sprinkled with sewage water to enhance bacterial population and moisture in the soil. The films were periodically taken out every 15 days and carefully wiped with tissue wipes. The film's weight was then recorded and films buried back in the soil. Weight loss was calculated as:

$$\text{weight loss (\%)} = \frac{w_0 - w_t}{w_0} \times 100 \%$$

where w_t and w_0 are weights of films after time t and initially respectively.

Graph of weight loss with time was plotted.