# ENZYME-ASSISTED ISOLATION OF MICRO FIBRILLATED CELLULOSE (MFC) FROM SACCHARUM MUNJA FIBRE AND ITS CHARACTERIZATION

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## ABSTRACT

**The use of unconventional renewable sources as raw material to produce micro fibrillated (MFC) cellulose is the alternative sought to maintain ecology. Saccharum Munja grass is a renewable grass which contains fibrous material which can be extracted as short fibres. These extracted fibres were used as raw material to isolate MFC using enzyme assisted ball milling process. The process parameters were optimised by varying the enzyme concentration and time period. Characterisation of the isolated MFC was carried out. The particle size of MFC was measured using particle size analyzer. 10 micron particle size was achieved of the MFC. Morphology of isolated MFC was studied using electron microscopy. Presence of the different chemical groups was analysed using infrared spectroscopy. X ray diffraction analysis, thermal gravimetric analysis (TGA) and Surface behavior of MFC was also studied. From the present studies it can be concluded that the isolation of smaller particle size MFC can be achieved by enzyme assisted ball milling without acid hydrolysis.**

**KEYWORDS:** Saccharum Munja, Enzyme, Ball Milling, Micro Fibrillated Cellulose

Saccharum Munja (S. Munja) is a highly drought and frost resistant, persistent grass with ecological importance found in arid areas and along river banks in India. The common name of the plant is Kana or Sarkanda or Munja. The geographical distribution of the S. Munja grass is between north and north-western parts of India and also found in Pakistan and Afghanistan. The grass is tall having height of 2 to 2.5 meters having silky and greenish brown panicles. Leaf sheath of grass contains fibres which can be extracted [Rahar et. al., 2001]. The extracted fibres have high cellulose content which can be processed into whisker-like micro fibrillated cellulose (MFC). MFC has varied applications in different fields as they are renewable, exhibiting a number of well-known advantages, such as low cost, worldwide availability, biodegradability, high stiffness, good mechanical properties and thermal recyclability by combustion. There are potential uses of micro cellulose within different industries are anti-caking agents, emulsifiers, binders, disintegrating agents, dispersants, emulsion stabilizers, thermal stabilizers, carriers for fast drying and dispersing agents. The diameter of MFC fibrils is usually at the range of 10–100 nm and can be up to several micrometres in length, depending on the preparation methods and material source [Qua et. al., 2011]. MFC is commercially produced using softwood pulp as source. MFC have been produced using various conventional fibres like cotton, jute, ramie etc. Different methods have been reported on the isolation of MFC from lingo-cellulosics. Work has also been reported for isolation of MFC from sisal fibres using treatment with sodium chlorite, followed by NaOH and acid hydrolysis. Wheat straw, jute fibre and soy hull were also used as cellulose source for preparation of MFC. Treatment was carried out by soaking in NaOH followed by acid hydrolysis and peroxide bleaching. Commercially MFC is produced by acid hydrolysis method using wood as raw material [Jahan et. al., 2011].

In the present work enzyme assisted ball milling method is proposed to produce MFC using a non conventional cellulosic (S. Munja) fibre as source. The S. Munja fibre is a renewable raw material for MFC production which gives cost advantage along with environmental benefits over the wood pulp used for MFC production conventionally. The proposed method is acid free which reduce effluent load in industry.

## EXPERIMENTAL

### Extraction of Fibres

The S. Munja grass was obtained from a village Narena Katta in east Rajasthan. The extraction of the fibres from grass was carried out as explained in the previous work. Briefly the grass was cut and dried and further the culms of the grass were separated. The bundle of culms was thrashed by wooden hammer to convert it into fibrous form. After thrashing, the retting of fibres was carried out using 2% NaOH for 72 hours. (Figure 1) Retted fibres were washed multiple times till the fibres were cleaned [Singh et. al., 2014].

### Pre-treatment of Fibres

Extracted fibres from S. Munja grass were preconditioned prior to the cellulose extraction. The fibres were washed with distilled water several times and dried in an oven at $80^0$C for 24 h**.** The pre-conditioned fibres were then chopped to an approximate length of 10-15 mm. The chopped fibres were treated

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with 5 M caustic solution at 80°C for 4 hours with continuous agitation followed by repeated washing to remove residual alkali. Further bleaching of fibres was done with 3g/l hydrogen peroxide solution at 10.5pH for 1 hour at 55 °C followed by repeated washing.

**Isolation of Micro Fibrillated Cellulose (MFC)**

Cellulase (Commercial Name: Cellusoft) enzyme was procured from Sarex Chemicals Ltd Mumbai. Pre treated fibres were soaked in different concentrations of enzyme solution at 45-55$^0$C for 5 mins. These fibres along with enzyme solution were transferred to ball milling machine for milling. The milling was done at 3000rpm using ceramic balls at 45-55$^0$C.

The optimization of the treatment condition was carried out. Milling of the fibres was carried out for various time periods and varied enzyme concentration. As the treatment time increased, the reduction in particle size was recorded. However, the increasing enzyme concentration did not have significance effect in particle size reduction during milling. 2 % enzyme concentration was optimised for further MFC production. Post milling, slurry of MFC was diluted with distilled water heated to deactivate enzyme. This solution was then centrifuged at 6000rpm for 10 min. The centrifuged solution is freeze dried to get MFC in powder form. Further, the characterisation of the powdered MFC is conducted.


**Figure 1: Isolation of MFC from S. Munja fibre**

**Characterization**

**Particle Size Analysis**

The MFC dispersion was prepared in water with the ratio of 1:100 (w/v) and ultra-sonicated for 5 min in an ultrasonic bath. Particle size was measured using Cilas 1064, France particle size analyser.

**Fourier Transform Infrared Spectrophotometer (FTIR) Analysis**

The FTIR spectra of MFC samples were recorded in the 4000 – 500 cm$^{-1}$region on a Shimadzu 8400S FT-IR instrument with 32 scans in each case at a resolution of 4 cm$^{-1}$.

**Thermo-gravimetric Analysis (TGA)**

The thermal behaviour of isolated MFC samples was analysed using SHIMADZU DTG-60H. Sample weighing 5mg was analysed under a nitrogen atmosphere with 100 ml /min of gas flow rate, heating rate of 10°C/min, and a temperature range from 25 to 500°C.

**X-ray Diffraction Analysis (XRD)**

The X-ray diffraction analysis of MFC samples was carried out on Shimadzu XRD 6100 at 40 kV and 30 mA. The experiments were performed in symmetrical reflection mode with Cu Kα radiation (λ = 1.5405 Å). Crystalline index (CI) was evaluated by Segal methods using Equations (1) where I002 is the largest intensity of the peak corresponding to the plane in the sample at 2θ angle in the range of 22◦ –23◦ representing crystalline material and Iam is the intensity of diffraction of the crystalline material, which is taken at an angle of about 18° 2θ in the valley among the peaks with minimum intensity representing amorphous material in the cellulose fibre.

$$ICr = \frac{I002 - Iam}{I002} \times 100 \quad \text{..... Eq. (1)}$$

The crystallite size (CS) of the MFC was determined by the following Scherrer's formula:

$$CS = K\lambda/\beta\cos\theta \qquad (2)$$

Where "K"=1 is Scherrer's constant, "β" is the peak's full-width at half-maximum and "λ" is the wavelength of the radiation [Terinte et. al., 2011].

**Scanning Electron Microscope (SEM)**

Surface morphology of isolated MFC samples were studied using JEOL Scanning Electron Microscope Model number JSM 5400, JEOL ltd. Japan.

## RESULTS AND DISCUSSION

**Particle Size Analysis**

The particle sizes were measured by light scattering method. This method cannot be related to the crystals diameter and length dimensions of their bundles. Figure 2 represents the particle size distributions of various ball-milled MFC samples for different enzyme concentrations. Slight reduction in

particle size was observed for the initial period between 1 – 2 hours but as the treatment time is increased the reduction in particle size was very significant. As the treatment time increased, the swelling of fibres occurred which caused easy access to enzyme to act on the fibres. The particle size of up to 8 – 10 µm was achieved after 24 hrs of treatment for irrespective of the concentration of enzymes. Thus it can be concluded that increasing concentration of enzyme does not have significant effect on particle size reduction. However as the time of treatment increased the particle size was reduced significantly.

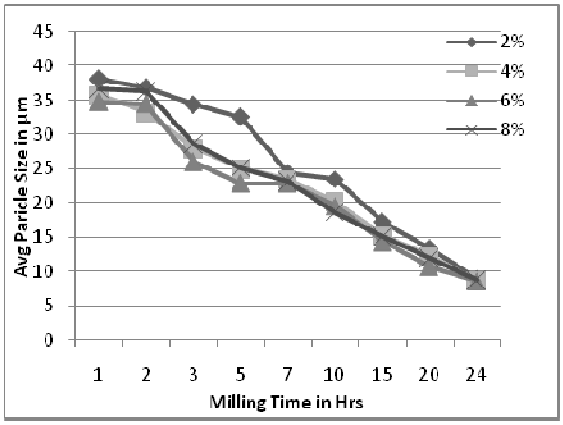


**Figure 2: Effect of Milling Time on Particle Size Of MCF**

**Fourier Transform Infrared Spectrophotometer (FTIR) Analysis**

Fourier Transform Infra-Red spectroscopy of various isolated MFCs was carried out (Figure 3). All samples showed the presence of two main transmittance regions; the first one at low wavelengths in the range 700–1,800 $cm^{-1}$, and the second one at higher wavelengths corresponding to the range 2,700– 3,500 $cm^{-1}$ approximately. In addition, due to the presence of functional groups such as -O-$CH_3$, C–O–C and aromatic C-C, peaks in the region between 1830 $cm^{-1}$ and 1730 $cm^{-1}$ were observed. There is no change in the functional groups during the treatment. Thus, FTIR spectra of the samples did not show any difference for the different MFC samples.

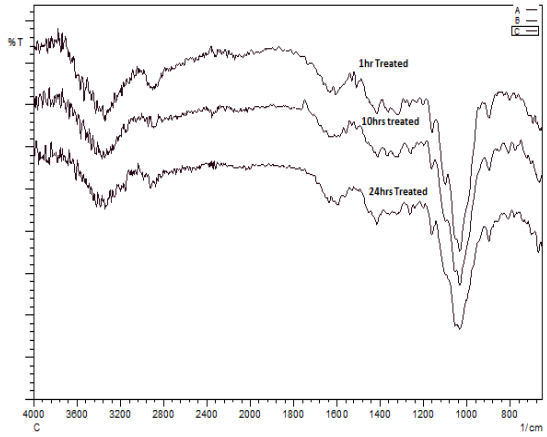


**Figure 3**: **FTIR spectra of Isolated MFC Analysis**

**X-ray Diffraction Analysis (XRD)**

The XRD patterns of ball-milled MFC samples are depicted in the figure 4. The intensity of the crystalline peak decreases with increasing time of treatment. The relative crystallinity index was also calculated for all samples using Segal's method. The crystallinity index of MFC reduced substantially from 64.1% for the 1 hour treated sample to 48.38% for the 10 hours ball-milled sample and 38.23% for 24 hours ball-milled sample. It is predicted that the cellulose structures are transformed into amorphous cellulose structures. The crystal size (CS) of 1hour treated sample was found 2.82 nm whereas CS of 10hours and 24hours treated sample was found 1.88nm and 1.41nm respectively. Mechanical ball milling of MFC decrease the particle size and CS and thus, increase the amorphous content in MFC. The hydrogen bonding in the cellulose is weakened due to the mechanical action during the ball milling. Thus there is decrease in crystallinity and amorphous cellulose content in MFC is higher than the fibre [Yu and Wu, 2011].

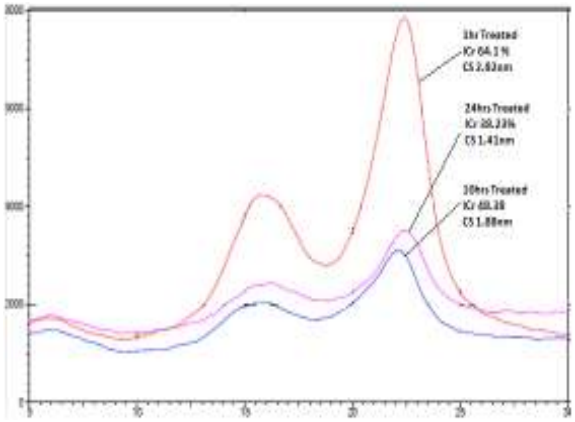


**Figure 4: X-ray Diffraction Analysis (XRD)**

**Thermo-gravimetric Analysis (TGA)**

The initial weight loss of MFC samples occurred below $100^0$ C regardless of their treatment. All the lingo-cellulosic fibres have hydrophilic character hence the initial weight loss occurs due to the vapourization of the moisture content. Further weight loss is due to the degradation of the cellulose in MFC [Johar et. al., 2012]. In 1 hour treated sample, 10% weight loss was observed at $206^0$C however, in samples where the treatment time was 10 hours and 24 hours, same weight loss was observed at $238^0$C and $240^0$C respectively. At higher temperatures, a sharper weight drop is observed. 50% weight loss was observed at 342◦C, 352◦C and 370◦C for 1 hour, 10 hours and 24 hours treated samples respectively. Thus the thermal degradation temperature increased as the treatment time increased, although crystalline index showed a decreasing trend as the treatment time increased. Thermal degradation temperature depends on the degree of polymerisation, orientation of molecular chains and the crystalline index of the material. The authors proposed a probability of the As the treatment time increases the effect of the shearing action during the mechanical agitation in ball milling increases. This may lead to increased orientation in amorphous region and thus the thermal stability.

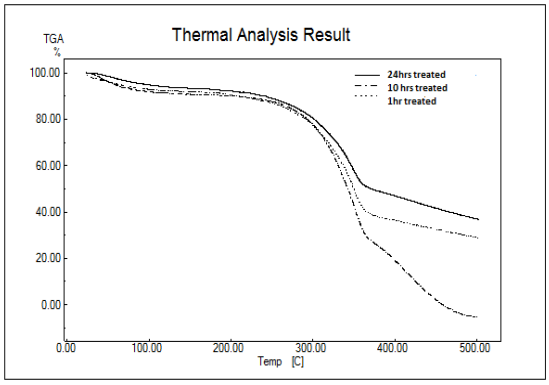


**Figure 5: Thermo-gravimetric Analysis (TGA)**

**Scanning Electron Microscope (SEM)**

The surface morphology of MFC was studied with a scanning electron microscope. The SEM images recorded are shown in figure 5. In the samples having the treatment time of 1 hour, it can be seen that MFC retains its rod like fibrous shape (Fig a. & b.). As milling time increased up to 10 hours and 24 hours MFC became irregular in shape. This can be attributed to mechanical action of ball milling. Due to ball milling the rod shaped micro cellulose is continuously exerted to mechanical stress and strain and hence become irregular shaped.

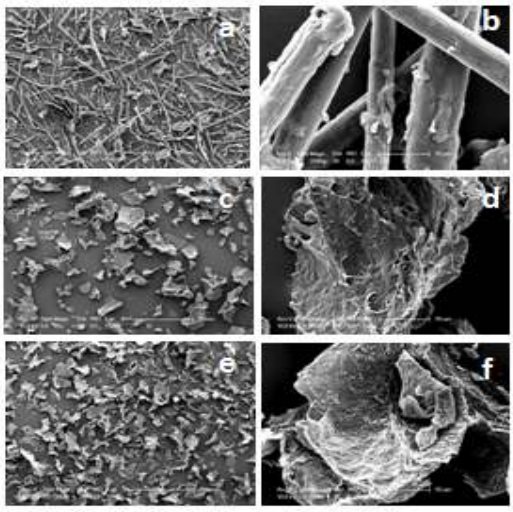


**Figure 6: a, b: 1hr treated MFC, c,d : 10hrs treated MFC, e,f: 24hrs treated MFC**

## CONCLUSION

The application of microfibers is extending in the various fields like medical, cosmetics, composite reinforcement and others. Most of the commercialized micro fibrillated celluloses are manufactured using tree wood as raw materials. Tree wood is a very valuable natural resource which is non-renewable. Due to the non-renewability the research focus is shifting towards the use of the renewable cellulose sources cotton, jute, flax, linen and other fibres however, these fibres have well established uses in the apparel and allied industries. In this work the novel source of fibres extracted from S. Munja grass, perennial in nature is used for the preparation of MFC. It is a very ecological and also economic source for the preparation of micro-cellulose. The treatment method used for the isolation of micro-cellulose from S. Munja fibres does not involve the use of any harsh chemicals that are involved in conventional acid hydrolysis methods, thus the replacement of unsustainable chemicals is also achieved.

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