CHAPTER – III

MATERIALS AND METHODS

The research was an experimental and exploratory study. The study was conducted to construct non-woven from extracted Sugarcane fibres under optimised extraction conditions, which can be used as a material for oil sorption and recovery from oil spills. This chapter deals with the materials used and methods followed to conduct the present investigation. It was divided into three major phases (Figure 3.1). The different methodological procedures adopted for the study have been described under the following heads:

3.1 Procurement of waste Sugarcane bagasse

3.2 Extraction of fibre from waste Sugarcane bagasse

3.3 Optimisation of fibre extraction process

3.4 Preliminary testing

  3.4.1 Physical properties of extracted fibre

  3.4.2 Chemical properties of extracted fibre

3.5 Fabrication of an apparatus (Sugarcane fibre extractor)

3.6 Modification of fibre through :

  3.6.1 Acetylation treatment

  3.6.2 Cyanoethylation treatment

  3.6.3 Enzymatic treatment

3.7 Optimisation of modification treatments

3.8 Characterisation of untreated and optimised fibre

  3.8.1 Physical characterisation through SEM analysis

  3.8.2 Chemical characterisation through FTIR analysis

3.9 Manufacturing of Non-woven

3.10 Testing of Non-woven for oil sorption

3.11 Analysis of data applying statistical tools

Research Design

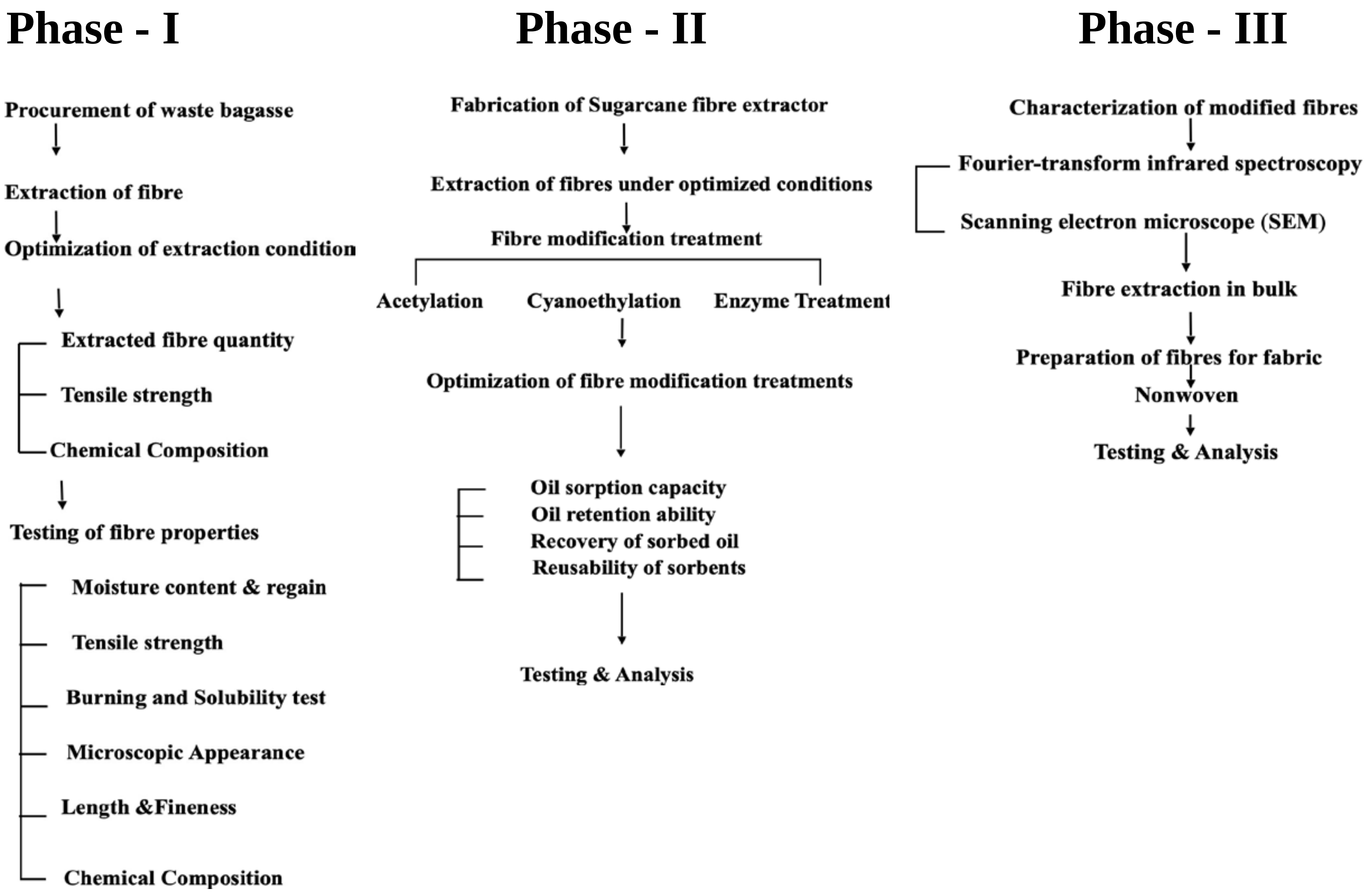


Figure 3.1: Flow chart of Research Design

3.1 Procurement of waste Sugarcane bagasse

In this study, waste Sugarcane bagasse (raw material) was procured from local juice vendors in Vadodara City, Gujarat, at an earlier stage of research. Later, after juice extraction, waste bagasse was procured from the Main Sugarcane Research Station, Navsari Agricultural University, Navsari, Gujarat, India.

3.2 Extraction of fibre from waste Sugarcane bagasse

Sugarcane bagasse is mainly composed of an outer rind and an inner pith. The softcore pith was removed from the bagasse manually. The outer rind was cut across the length; nodes were removed and separated from all the pithy material. The staple rind was washed with hot water (material: liquor ratio1:50) at 90℃for 60 minutes to remove the sugar traces, dirt and dust particles. The washed material was treated with varying concentrations of sodium hydroxide (NaOH) viz 0.1 (N), 0.2(N), and 0.3 (N) at 90℃at different time durations viz 60,120, 180 and 240 minutes, with vigorous stirring. The treatment was followed by hot water washing and subsequently neutralised with dilute (10% dilution) hydrochloric acid solution. The extracted material was further thoroughly washed with cold water and dried in condition. Extraction of fibres was carried out in the Textile Chemistry Laboratory, Department of Clothing and Textiles, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India.

3.3 Optimisation of the fibre extraction process

To optimise parameters (chemical concentration and time) for the fibre extraction process, sodium hydroxide (NaOH) solution in three different concentration of 0.1 (N), 0.2 (N) and 0.3 (N) was taken with the time duration of 60 min, 120 min, 180 min and 240 min, for each concentration respectively. The above three parameters were analysed to optimise the extraction process. The treated materials were statistically analysed with the help of a full factorial design of the experiment for the following response variables:

3.3.1 Fibre quantity

3.3.2 Chemical composition

3.3.3 Tensile strength

3.4 Preliminary testing

3.4.1 Physical properties of extracted fibre

3.4.1.1 Burning test

To recognise the composition of fibres by burning test, the fibre was moved slowly towards a small flame, and the reaction to heat was observed. One end of the sample fibre was put directly into the flame to determine its burning rate and characteristics. The burning odour was noticed.

3.4.1.2 Fibre morphology

The longitudinal and cross-sectional characteristics of fibre were studied by microscopic analysis. The longitudinal section and the cross-sectional view (Plate 3.2) of the fibre were observed under a polarized light microscope (Plate 3.1) with a magnification of 45 X power in the Textile Testing Laboratory of the Department of Clothing and Textiles, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India.

The Fibre cross-section view from magnification of 50 X to 2500 X power was also observed with SEM (JEOL JSM IT 200) (Plate 3.3) at Bombay Textile Research Association (BTRA) Laboratory, Mumbai, India. For a cross-section view of the fibre, the bundle of fibres was passed through the tube and fixed compactly. Then, the fibre-filled tube was sharply cut into small pieces and placed on the plate after coating. Carbon tapes were used to mount the rest of the area to avoid charging, the specimen was scanned using suitable magnification, and micrographs were recorded.

Plate 3.1: Polarized light microscope

Plate 3.2: Slide for fibre cross-section

Plate 3.3: Sample preparation unit for SEM testing in BTRA laboratory

3.4.1.3 Fibre length

Fibre length was measured using a steel ruler. Each fibre was gently kept on the ruler, and the readings between both ends were recorded. A total of 50 readings were taken, and the average was calculated to determine the fibre length.

3.4.1.4 Fibre diameter

Fibre diameter was viewed under a polarized microscope using a 10 X micrometre lens. A total of 50 readings were taken, and the average was calculated to determine the diameter of the fibre.

3.4.1.5 Fibre fineness

The fineness (denier) of the fibre was determined using the ASTM D 7025-09 test method. An average of 20 observations of 100 mm length of fibre was taken and then calculated using the formula given below:

$$\text{Denier} = \frac{W \times l}{L} \qquad \text{……………} (1)$$

Where, W = Weight of the fibre
L = length of the sample
l = the unit length of the system

3.4.1.6 Fibre moisture content and regain

To determine the moisture content and regain, the fibres of known weight were conditioned at 65±2 % RH at 27±2 °C temperature. The samples were weighed in a conditioned state (W) and then dried at 110 °C in the oven. After an hour, samples were taken out of the oven and weighed (D). The difference in weight, moisture content, and moisture regain was calculated using the following equation (Booth,1996).

$$\text{Moisture content (M) (\%)} = \frac{100\,W}{D+W} \qquad \text{…………………}(2)$$

$$\text{Moisture regain (R) (\%)} = \frac{100\,W}{D} \qquad \text{…………………}(3)$$

Where D = Oven dry weight
W = Weight of Water
M = Moisture content
R = Regain

3.4.1.7 Fibre tensile strength

Single fibre strength tests for sugarcane fibre specimens were carried out on Llyod Instron Tensile Testing Instrument (ASTM D 3822) in the Textile testing laboratory, Department of Textile Engineering, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara. This instrument works on the principle of constant rate of extension (C.R.E.). A test single fibre was done in which fibre of 10 cm length was fixed between a pair of jaws, the bottom jaw was fixed, and the upper jaw was movable; it moved with a constant speed of 5mm/min and gauge length 100 mm.

Plate 3.4: Tensile strength tester

Test Specimens having gauge lengths of 100 mm were used. An average of 20 readings were taken for each sample. So, a total of 200 readings were taken.

3.4.2 Chemical properties of extracted fibre

3.4.2.1 Chemical solubility test

To examine the solubility of fibre, the fibres were subjected to different alkali and acids in both cold and hot conditions at various concentrations.

3.4.2.2 Chemical composition

The chemical composition of the fibre was determined as per the scheme suggested by Garner (1967), with the help of the soxhlet apparatus (Plate3.5). The procedure was carried out in the following sequence:

a) Estimation of water-soluble components

A weighed sample of known moisture regain was boiled in distilled water for five hours, using a liquor ratio of 1:30. Sample was filtered in a sintered glass crucible of no: 1 porosity, oven-dried at 100 ℃ for 30 minutes, conditioned at 65±2 % RH at 27±2 ℃ temperature and weighed on an electronic balance. The water-soluble compounds were expressed as a percentage of the oven-dry weight of the original sample and were calculated using the following equation:

Water soluble components (%) = W_1-W_2 / W_1×100 .... (4)

Where W_1 = Initial weight of the sample

W_2 = Weight of the sample after the procedure (removal of water-soluble)

b) Estimation of fats and wax content

After the removal of the water-soluble component from the sample, it was extracted in the soxhlet apparatus with 2:1 alcohol (methanol): benzene for four hours. The sample was then washed with alcohol, oven-dried, and weighed (W_3 grams). The result was expressed as a percentage of the oven-dry weight of the original sample and was calculated using the following equation:

Fats and waxes (%) = W_2-W_3 / W_1×100 .... (5)

Where W_2 = Initial weight of the sample

W_3 = Weight of the sample after the procedure (removal of fats and waxes)

c) Estimation of pectin content

The defatted fibre sample was then extracted by boiling for one hour in a 1% ammonium oxalate solution and washed with distilled water until the washings were free from oxalate. The loss in weight owing to the removal of pectinous material is recorded as a percentage of the oven-dry weight of the original sample using an equation.

Pectin content (%) = W_3-W_4 / W_1×100 .... (6)

Where W_3 = Initial weight of the sample

W_4 = Weight of the sample after the procedure (removal of pectin content)

d) Estimation of hemicellulose content

After the pectin removal, the fibres were extracted in the soxhlet apparatus with 2% caustic soda solution for one hour and then washed thoroughly with distilled water. The loss in weight due to the removal of hemicellulose is estimated as a percentage of the oven-dry weight of the original sample using the equation:

Hemicellulose content (%) = W_4-W_5 / W_1 ×100 ....(7)

Where W_4 = Initial weight of the sample

W_5 = Weight of the sample after this procedure (removal of hemicellulose content)

e) Estimation of lignin content

The above-treated samples were treated for two hours under reflux in a water boiling bath with a 50:1 liquor ratio of 0.7% sodium chlorite solution at 4 pH. The pH was obtained by adding acetic acid to the solution. This treated sample was filtered in a sintered glass crucible of number one porosity. Later the samples were washed with 750 ml of distilled water, then with 250 ml of 2% sodium bisulphite solution, and then finally with 1000 ml of distilled water. Later, the samples were dried at 105 ℃. The lignin content as a percentage of the oven dry weight of the original sample was calculated by:

Lignin content (%) = W_5-W_6/ W_1 ×100 ....(8)

Where W_5 = Initial weight of the sample

W_6 = Weight of the sample after the procedure (removal of lignin content)

f) Estimation of cellulose content

After the removal of non-cellulosic components, cellulose was measured by weight differences using the equation:

Cellulose content (%) = W_6/ W_1×100 ....(9)

Where W_6 = Weight of the sample after the lignin removal.

W_1 = Initial weight of the sample

Plate 3.5: Soxhlet apparatus

3.5 Fabrication of an apparatus (Sugarcane fibre extractor)

The important factor for the extraction of fibre was continuous heating with stirring. Initially, the experiment was performed in the laboratory using a REMI lab stirrer (Plate 3.6) with high RPM (500-900) and sharp blade edges, which led to entanglement and crushing of the material and consequently stopped the stirring operation.

Plate 3.6: Extraction experiment with lab stirrer

Therefore, an apparatus (Sugarcane fibre extractor) was designed and fabricated to extract fibre efficiently from the outer rind of Sugarcane bagasse.

3.6 Modification of fibre through chemical and enzymatic treatments

Sugarcane bagasse fibre has been modified using Acetylation, Cyanoethylation, and Enzymatic treatments to enhance its oil sorption capacity. These chemical modifications can reduce hydrophilicity of cellulose or, in other words, improve its oleophilicity. Natural fibres surface treatment is essential to reducing the hydrophilicity of the fibres. The codes were assigned for untreated and treated fibre samples given in Table 3.1.

Table 3.1: Coding of the untreated and treated Sugarcane bagasse fibres

S.No.	Fibre Codes	Description
1	UB	Untreated bagasse
2	AC	Acetylation treated
3	CY	Cyanoethylation treated
4	EZ	Enzymatic treated

3.6.1 Acetylation treatment

The acetylation method is very effective in modifying the surface of natural fibres to increase their hydrophobicity. Acetylation refers to the process of introducing an acetyl group $[(CH_3\ CO)_2 O]$ into a compound, namely the substitution of an acetyl group for an active hydrogen atom. Generally, the treatment involves applying acetic anhydride and acetic acid to plasticise the cellulosic fibres (esterification method). The esterification occurs by the reaction of acetyl groups (CH_3CO–) with hydroxyl groups (–OH) on the fibres.

The hydroxyl groups are there after replaced by a hydrophobic acetyl group (CH_3CO) inducing the change of the hydrophilic nature to the hydrophobic nature of the fibre. Acetylation significantly increased the hydrophobic properties of the bagasse.

Acetylation using perchloric acid ($HClO_4$) as a catalyst

The grey fibres were initially immersed in glacial acetic acid for one hour, followed by acetylation using acetic anhydride, diluents and catalysts for different temperature and time. Then, fibre was thoroughly washed with water and dried. Perchloric acid was used as a catalyst, and glacial acetic acid was used as a diluent in the study. The recipe for acetylation using $HClO_4$ as a catalyst is given in Table 3.3.

Table 3.2: Composition of perchloric acid and acetic anhydride

Perchloric acid ($HClO_4$)(ml)	**Acetic anhydride($(CH_3CO)_2O$ (ml)**
1	99
3	97
5	95

Table 3.3: Recipe for Acetylation using perchloric acid as catalyst

Parameters	**Conditions**
Catalyst mixture	15 ml
Glacial acetic acid	85 ml
Liquor ratio	1:30
Temperature	30℃, 50℃ & 70 ℃
Time	30, 60 & 90 minutes

Procedure:

The grey fibres were initially immersed in glacial acetic acid for one hour, followed by acetylation. For acetylation, perchloric acid was taken and mixed in acetic anhydride (catalyst mixture) – Solution ‘A’. Then,15 (ml) of the catalyst solution 'A' was taken, and 85 ml of glacial acetic acid was added to it. Material: Liquor ratio = 1:30 was taken. The Care was taken to ensure that the sample was properly dipped in the solution. The sample was heated at different temperatures in a water bath with continuous stirring for 30, 60, and 90 minutes. Then, liquor was removed, and fibres were taken in a beaker and neutralised. After treatment, sodium carbonate solution (4%) was prepared to neutralise the sample. The samples were thoroughly washed with cold water and finally washed with ethanol and dried. Oven dried at 60-70 ℃for 30 minutes.

3.6.2 Cyanoethylation treatment

Cyanoethylation is an etherification treatment that has two steps. The fibre is first pre-treated with sodium hydroxide (NaOH), and then the pre-treated sample is reacted with acrylonitrile (C_3H_3N), which is called Cyanoethylation.

Acrylonitrile (C_3H_3N) is an organic compound that is constructed primarily of carbon and hydrogen atoms. It is a relatively small compound containing only three carbon and three hydrogen atoms. It also has a nitrogen atom along with the carbon and hydrogens. Acrylonitrile has two important functional groups, one being an alkene and the other a nitrile. Cyanoethylation is the reaction of introducing a nitrile group ($C \equiv N$) [i.e., also called cyano group] with an ethyl group into the fibre structure. The recipe for the cyanoethylation treatment are presented in Table 3.4.

Table 3.4 Recipe for Cyanoethylation treatment

Parameters	**Conditions**
NaOH concentration (w/v)	1,5 & 9%
Acrylonitrile	99%
Liquor ratio	1:30
Temperature	30℃, 50℃ & 70℃
Time	30, 60 & 90 minutes

Procedure

The grey fibre was treated with different Conc. of NaOH solution for 10 minutes and then reacted with acrylonitrile at varied time and temperature with continuous stirring in a water bath. The treated samples were first washed with hot water, neutralised using dilute acetic acid and then washed with cold water, finally washed with ethanol and dried. The treatment was carried out in two steps:

i) **Pre-treatment:** The fibre is first pre-treated with sodium hydroxide (NaOH). The grey fibre sample was taken and treated with a NaOH solution (M: L = 1:30) for 10 minutes at room temperature with continuous shaking under a water bath. Treatment with NaOH makes the fibre readily accessible to the modification reagent.

ii) **Reaction with acrylonitrile (C_3H_3N):** Pre-treated fibre is treated with acrylonitrile (C_3H_3N). The fibre sample was taken out from the NaOH solution, squeezed, and treated with acrylonitrile (M: L = 1:30) for different treatment time and temperature with continuous stirring under a water bath. After the treatment, the fibre sample was washed with hot water and cold water, respectively, for neutralisation. The neutralised fibre sample was dried in a hot air oven at 60-70℃for 30 minutes.

3.6.3 Enzymatic treatment

Two enzymes, cellulase and pectinase, were used for the study. Both enzymes were procured from Rossari Biotech Ltd., Ahmadabad, Gujarat. Cellulase and pectinase were used in an equal ratio (1:1). The recipe for enzymatic treatment is given in Table 3.5.

Table 3.5 Recipe for Enzymatic treatment

Parameters	**Conditions**
Cellulase & Pectinase (1:1)	1,5 & 9%
Liquor ratio	1:60
Temperature	30, 50 & 70 ℃
Time	30, 60 & 90 minutes
pH	5-6

Procedure

The grey fibre sample was treated with enzyme (M:L = 1:60) at different treatment time (30, 60, 90 minutes) and temperature (30, 50, 70℃) with continuous stirring in a water bath (Plate 3.7). After the treatment, the fibre sample was washed with cold water to neutralise the fibre sample. The washed fibre sample was dried in a hot air oven at 60-70℃for 30 minutes.

Plate 3.7: Water bath with shaker in laboratory

Plate 3.8: Researcher performing an experiment in the laboratory

Plate 3.9: Digital weighing balance and oven in laboratory

3.7 Optimisation of modification treatments

After each treatment modification, the modified fibre samples were tested for their oil sorption capacity (ASTM F726-99), recovery of oil, oil retention and reusability of the sample (Bazargan et al., 2015).The standard test methods for testing of oil are mentioned below:

Testing of modified fibres for oil

i) Test for oil sorption capacity (ASTM: F726-99)

1 gm of fibre sample was immersed in 50 gm of oil in a glass beaker for 30 minutes. Then, the samples were hanged vertically for 15 minutes to drip the excess oil in the same beaker. The weight of oil collected after dripping was measured and used to calculate the oil sorption capacity. The oil sorption capacity was calculated using the following expression.

$$Q = \frac{a - b}{a} \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(10)}$$

$$\boxed{\frac{\text{Initial weight of oil} - \text{Final weight of oil}}{\text{Initial weight of oil}} \times 100}$$

where, Q = Oil sorption capacity

a = Initial weight of oil in gm (50 gm)

b = Final weight of oil

ii) Oil retention ability

A sample of 1 gm was placed in 150 ml oil for 15 minutes. The sorbent was then removed and hanged vertically, allowing the absorbed oil to drip from the sample. The weight of the material was measured after 15, 30, 45, 60 and 90 minutes of draining. The amount of oil retained was determined as the difference between the weight of the material before and after the stipulated draining period.

iii) Recovery of sorbed oil

After oil sorption, the sample was passed between two rollers of padding mangle (Plate 3.10) at a pressure of 98 N/cm^2. The amount of recovered oil was determined from the difference in weight of the sample before and after pressing.

iv) Reusability of sample

The pressed sample was again used in the sorption process. The efficiency of sample's reusability as oil absorber was determined by oil sorption capacity of the sample after repeated sorption and desorption cycles.

Plate 3.10: Padding mangle in laboratory

Plate 3.11: Researcher performing an experiment with Padding mangle in laboratory

The average readings were recorded for further optimisation of treatment parameters. All three modification treatments were optimised with the help of statistical analysis, viz. Response surface design of experiment. A Response Surface Design is a set of advanced design of experiments (DOE) techniques which provide a better understanding and optimise conditions for a response.

3.8 Characterisation of untreated and optimised fibre

3.8.1 Physical characterisation through SEM analysis

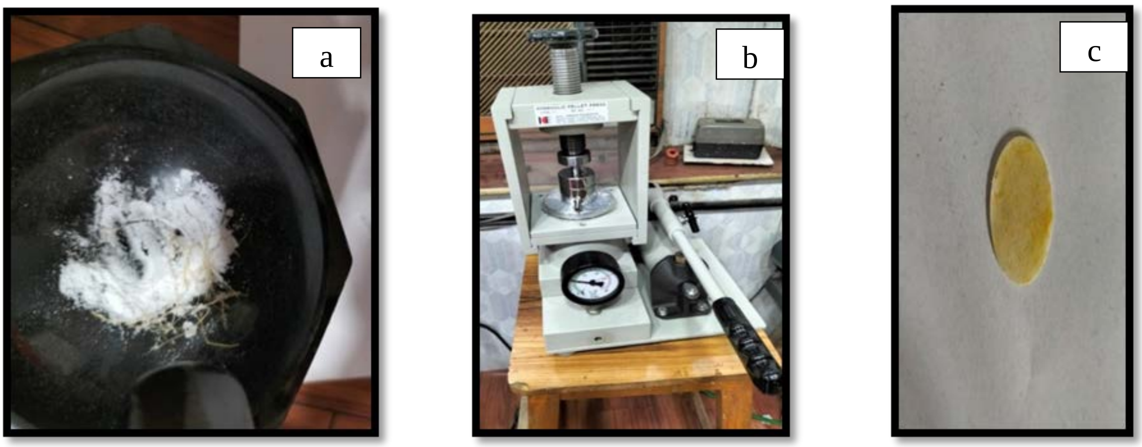
The scanning electron microscope (SEM) imaging system, with its wide range of magnification and great depth of focus, is remarkably well suited to the needs of textile technology for three main reasons. Firstly, fibres are small but are not microscopic and, hence, may be imaged easily and quickly. Second, fibre features that determine performance in manufacturing and conversion to end-use are of the same size range and are easily imaged.

The surface morphology of the untreated and optimised fibre samples was analysed using a scanning electron microscope, SEM (Joel JSM- 5610V). The fibre sample was mounted on an SEM Stub of 12mm X 12mm diameter using carbon tabs. The samples other than metal are polymeric materials, which are very sensitive to electron beams and get charged in electron beams. The charging of the specimen causes artifacts (astigmatism) and also focuses problems in the SEM. To avoid charging, the specimen was then coated with a thin layer of conducting material such as gold or gold-palladium using a Sputter Coater for one minute. After coating with Au/Pd in a sputter coater, the specimen was then scanned in SEM (Plate3.12). Using suitable magnification and accelerating voltage, micrographs were recorded. The test was conducted under the supervision of Dr. Vandana Rao, an expert in SEM from the Department of Metallurgical and Material Engineering, Faculty of Technology and Engineering (DST, PURSE, New Delhi), The Maharaja Sayajirao University of Baroda, Vadodara.

Plate 3.12: SEM Setup

3.8.2 Chemical characterisation through FTIR analysis

The FTIR analysis of the untreated and treated fibre samples was carried out in Borex Alpha–IR Spectroscopy (Plate 3.13) over the wavelength of 400 to 4000 cm^{-1}.The sample was prepared by freezing the chopped untreated and optimised fibres in liquid nitrogen and pulverizing it to yield a fine powder capable of being cast into traditional KBr pellets for IR analysis. The KBr pellets of samples were prepared by mixing 2 mg of fibre sample, finely grounded, with 200 mg KBr (FT-IR grade) in a mortal and pastel. The 13 mm KBr pellets were prepared under vacuum in a standard device (Hydraulic pellet press) under a pressure of 75 kN cm^{-2} for 3 minutes. For the analysis, the scanning range was from 400 to 4000 cm^{-1}. The spectrum was recorded in transmittance mode by using the ATR sampling technique. The test was conducted in the laboratory of the Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara.



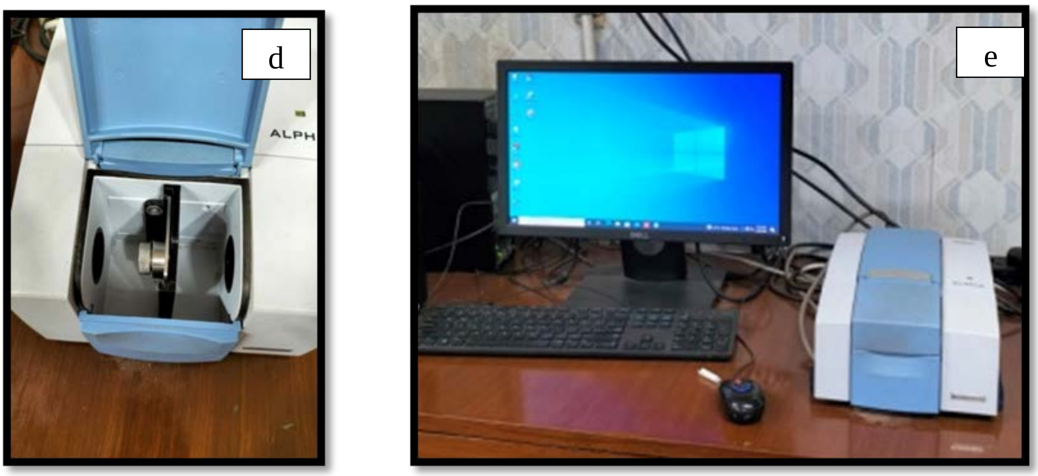


Plate 3.13: FTIR Setup (a) Sample preparation (b) Hydraulic pellet press (c) KBr pellet (d) Sample holder (e) Setup for spectra graph

3.9 Manufacturing of Non-woven

Non-woven structures were created from extracted fibres. The process involved cleaning and carding for the preparation of the fibre web and needle-punching as the bonding of the fibres. The Non-woven were prepared in a random-laid direction through mechanical bonding using a DILO-Needle punching machine (Plate 3.14). Web formation was done on the miniature carding machine (roller and clearer card for long staple fibres) for bast fibres. This machine has a feeding passage and a cylinder with a series of sharp nails projecting from its periphery. The nails help further open the exposed disintegrated fibre coming from the feed passage, remove the pith, and throw the cleaned fibres out. The process was carried out in the Laboratory, Department of Textile and Fibre Engineering, Indian Institute of Technology (IIT), Delhi, India.

Plate3.14: Needle-punching machine (laboratory at IIT Delhi)

3.9.1 Determination of fabric weight per unit area (GSM)

Specimen of non-woven 5 × 5 cm was cut and weighed. An average of five observations was taken. Using ASTM Test Method D3776-96, GSM was calculated with the following formula:

$$GSM = \frac{\text{Weight in grams of sample} \times 100 \times 100}{5 \times 5} \quad \text{…………(11)}$$

3.10 Testing of Non-woven for oil sorption

The prepared non-woven was tested for its oil sorption capacity, oil recovery, oil retention ability, and reusability in three different kinematic viscosities of oil: motor oil (25W-40), light viscosity crude oil, and heavy viscosity crude oil. The oil specification is presented in Table 3.6.

Table 3.6: Specification of oil used for testing

S.No.	**Specification**	**Motor oil** **(25 W-40)**	**Low-viscosity crude oil**	**High-viscosity crude oil**
1	Density(g/cm^3)	0.875	0.81	0.93
2	Kinematic Viscosity (mm^2/s)	121	352.3	623.3
3	Specific gravity(15℃)	0.82	0.81	0.93

The procurement of oil was done from Oil and Natural Gas Corporation Limited (ONGC), Vadodara, India. The specification of oil used in this study was taken as suggested by ONGC laboratory scientists as a practical approach for oil spill solutions. The codes were assigned for different oils used for testing (Table 3.7).

Table 3.7: Coding for oil tested in different viscosities

Sr.N0.	Codes	Description
1.	Mo	Motor oil (25W-40)
2.	LvCo	Low-viscosity crude oil
3.	HvCo	High-viscosity crude oil
4.	HvCoW	High-viscosity crude oil with a water layer
5.	HvCoW	High-viscosity crude oil with a water layer

3.11 Analysis of data applying statistical tools

The data collected through experimental procedures for fibre extraction parameters, modification and optimisation of treatments was coded, tabulated and analysed by the application of statistical tools as given below:

Design of Experiment (DOE): A response surface design is a set of advanced design of experiments (DOE) techniques which provide a better understanding and optimise conditions for a response. A full factorial design plot was taken for analysis. The Quadratic design model predicted by ANOVA was built, and the results were checked at a 0.05 level of significance.