Chapter 4

Material and methods

4.1 Introduction

This chapter discusses the experimental procedures and fully describes the raw materials used to synthesize porous metallic and ceramic scaffolds. A variety of contemporary characterization techniques are covered in this chapter as well, including energy-dispersive X-ray spectroscopy (EDAX), transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, and selected area electron diffraction pattern (SAED). The sequence and flow chart of the various raw materials utilized to fabricate green and sintered scaffolds are shown in Fig. 4.2.

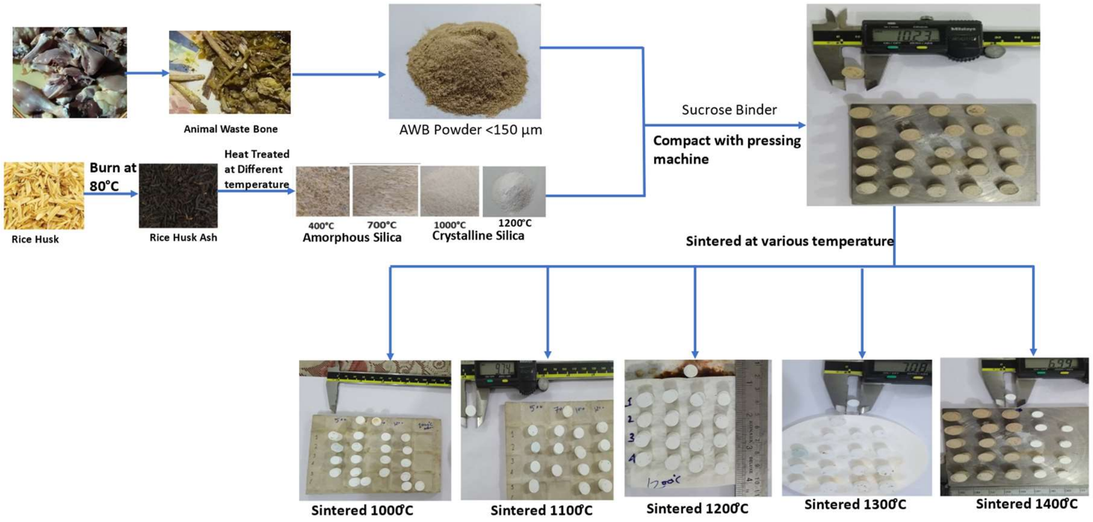


Figure. 4.1 Flow chart of waste material till sintered at different temperatures.

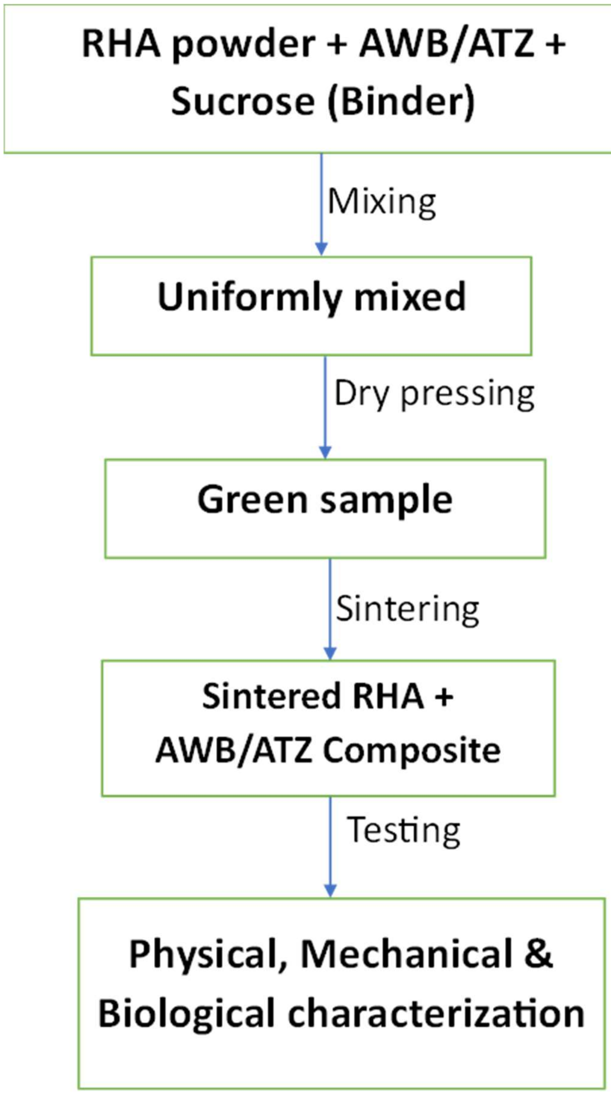


Figure. 4.2 Diagram showing the order in which the raw materials are processed.

4.2 Material used

The primary components utilized in the production of porous composites are Sucrose Solution (SS), Animal Waste Bone (AWB), and Rice Husk ASH (RHA) powder. The RHA and AWB powder were manually purified, and all of the materials utilized were of analytical grade. The process of converting residual RH into RHA powder of varying mesh sizes is illustrated in Fig. 4.1. The bone powder utilized in this investigation was generated from surplus bone from butcher shops and restaurants. The waste bone was extensively washed using deionized water to remove any unwanted materials, such as bone marrow and spices. In order to decrease the washed bone, it was simmered with common salt for two hours. Next, it was dried and crushed in the oven and a grinder. Figures 4.3, 4.4, and 4.5 illustrate the images of RH, RHA powder, and AWB powder, respectively.

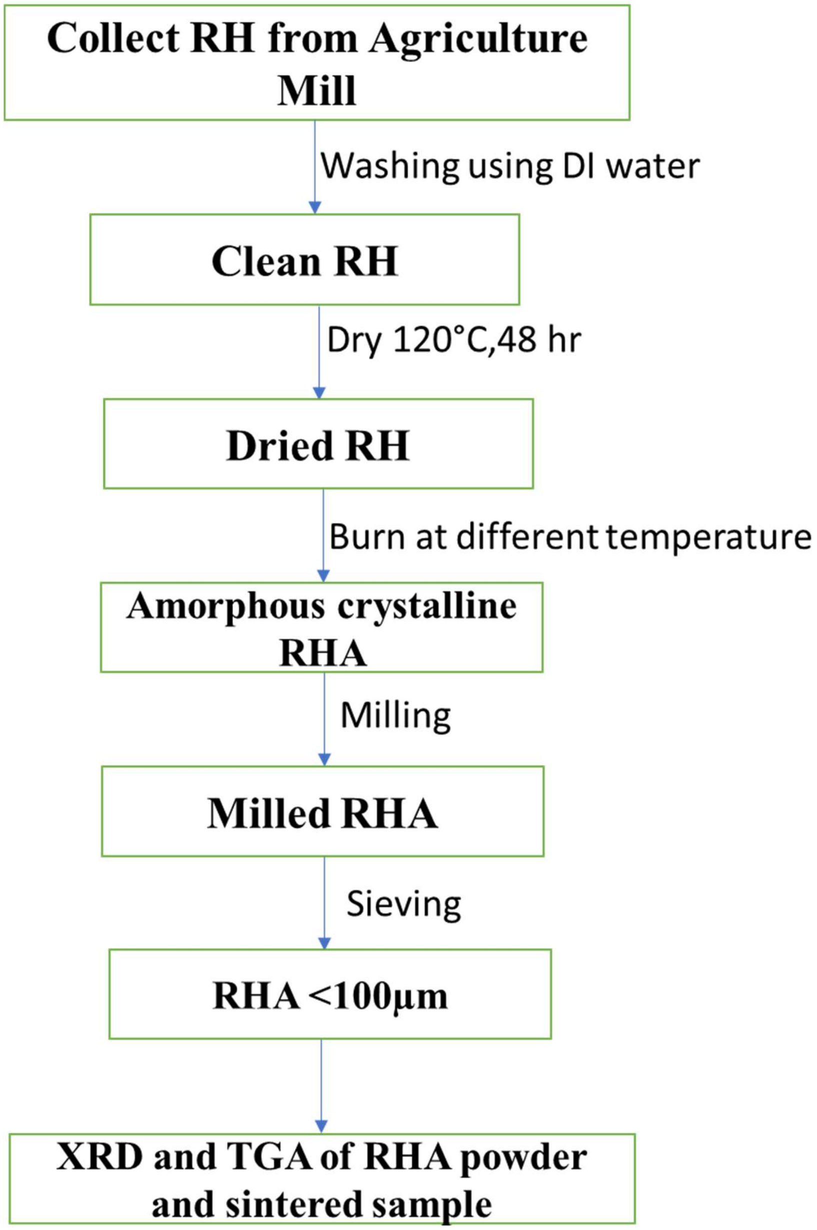
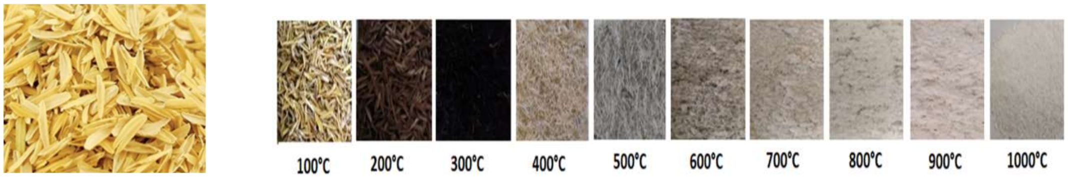


Figure. 4.3 Process flow diagram showing the Rice Husk (RH)-to-rice Husk Ash (RHA) powder processing.



(a) (b)

Figure. 4.4 (a) Thoroughly washed Rice Husk and (b) processed amorphous and crystalline Rice Husk powder

(a) (b)

Figure. 4.5 (a) Raw bone (b) Bone powder

4.3 Instrumentation

Several advanced characterization approaches were used to characterize the porous composite scaffold as prepared, and they are briefly explained here.

4.3.1 X-ray diffraction (XRD)

X-ray diffraction (XRD) is an advanced technique used to analyze the structure of crystals and measure the distance between their atomic planes. The fundamental idea relies on the constructive interference of monochromatic X-rays and a crystalline sample. The X-ray beam is produced by a cathode ray tube, filtered to provide radiation of a single wavelength, collimated to focus the beam, and then directed toward the sample. When the incident rays come into contact with the sample, they create constructive interference and a diffracted ray if the conditions meet Bragg's Law (equation 4.1).

The equation (4.1) represents the relationship between the wavelength (λ), the distance between two slits (d), and the angle (θ) at which the light is diffracted.

$$n\lambda=2d\sin\theta \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 4.1$$

In this context, n is an integer value, λ denotes the wavelength of X-rays, d represents the interplanar spacing that produces the diffraction, and θ is the angle at which the diffraction occurs.

Bragg's law establishes a connection between the wavelength of electromagnetic radiation, the diffraction angle, and the lattice spacing in a crystalline sample. Subsequently, the X-rays that have undergone diffraction are recognized, subjected to processing, and quantified. By systematically varying the 2θ angles while scanning the sample, all potential diffraction directions of the lattice can be achieved as a result of the random arrangement of the powdered material.

An X-ray tube, a sample box, and an X-ray detector are the three main parts of an X-ray diffractometer. In a cathode ray tube, X-rays are made by heating a filament to produce electrons, adding a voltage to speed the electrons towards a target, and then bombarding the target material with electrons. Characteristic X-ray spectra are made when electrons have enough power to knock out the inner shell electrons of the target object. These spectra have several parts, but K± and K² are the most common. Part of K± is made up of K±1 and K±2. K±1 has a wavelength that is a little shorter than K±2's and has twice as much power. These bands are unique to the target material (Cu, Fe, Mo, or Cr). To get the monochromatic X-rays needed for diffraction, foils or crystal monochrometers must be used for filtering. Because K±1 and K±2 have wavelengths that are close enough to each other, a weighted sum of the two is used. Copper (CuK± radiation = 1.5418É) is the most popular material used for single-crystal diffraction. The sample is pointed at by collimating these X-rays. The strength of the reflected X-rays is measured as the sample and detector are turned. The Bragg Equation says that constructive interference and a rise in intensity happen when the shape of the X-rays hitting the sample is right. This X-ray signal is picked up by a detector, which then processes it and turns it into a count rate that is sent to a printer or computer viewer. In Figure 4.6, you can see a basic drawing and a picture of XRD.

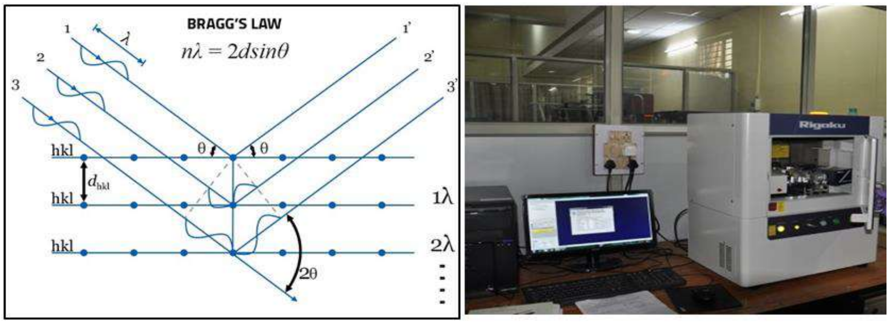


Figure. 4.6 A schematic representation of the basic principle of X-ray diffraction spectroscopy (XRD) and a photograph of the XRD machine.

4.3.2 Scanning Electron Microscopy (SEM).

One can acquire the surface morphology of a sample at various magnifications, resolutions, and depths of focus using a scanning electron microscope. Photographs are obtained with a better resolution than using an optical microscope. On the supplied solid surface is incident a well-focussed monoenergetic electron beam. Different scattering mechanisms are the outcome of the interaction between the beam and the surface. Surface morphology in SEM is mostly obtained by use of secondary electrons and backscattered electrons. The cathode ray tube (CRT) or monitor screen brightness is controlled by amplifying current signals generated by this BSE or SE. The conditions under which SEM operates are vacuum. Therefore, certain sample preparation is necessary before submitting the sample for imaging. Moisture content in the sample or the chamber should be avoided as it would prevent the chamber from creating a vacuum. Metals need no preparation before use because the sample should be conducted naturally. In order to make ceramic samples surface conductive, hin gold is later applied. The present work includes surface morphology examination by SEM of all sintered samples. The instrument model INSPECT 50 FEI was used for the SEM. Model: Carl Zeiss Microscopy LTD. EVO-scanning electron microscope MA15/18. The energy dispersive spectroscopy (EDX) on this SEM is equipped. To obtain the elemental analysis of the prepared samples, EDX and mapping were recorded at particular magnification.

Figure. 4.7 Setup of the scanning electron machine.

4.3.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is a method employed to identify various functional groups in organic and inorganic molecules or substances. It is a potent instrument for discerning a molecule's many types of chemical bonds by generating an infrared absorption spectrum that closely resembles a unique molecular "fingerprint". Fourier Transform Infrared Spectroscopy

(FTIR) is a technique that involves collecting and converting data from an interference pattern to a spectrum. The operating principle is based on the Michelson interferometer, which comprises a beam splitter, a stationary mirror, and a movable mirror that translates accurately in both directions. The radiation emitted from the source impinges against the beam splitter and undergoes a division process, forming two distinct beams. One beam is sent via the beam splitter and travels to the stationary mirror, while the second beam is bounced off the beam splitter and directed toward the moving mirror. The stationary and mobile mirrors redirect the radiation toward the beamsplitter. Once again, half of the reflected radiation is transmitted, while the other half is reflected at the beam splitter. This leads to one beam passing through the sample and being detected by the detector while the spectra are displayed on a computer. The second beam returns to the source. In our investigation, we conducted the FTIR spectra of the sample using the PerkinElmer Spectrum 100 instrument. The radiation sources passed through a KBr window, and a LiTaO3 detector communicated the gathered data. The resulting spectra were displayed in transmission mode. The sample pellets were created by combining the sample with KBr at a ratio of 1:100. These pellets were then analyzed by scanning them in the region of 400-4000 cm-1, with a spectral resolution of 4.0 cm-1 and a scan speed of 0.2 cm/sec. Figure 2.6 provides the operating concept and photograph of the FTIR spectrophotometer.

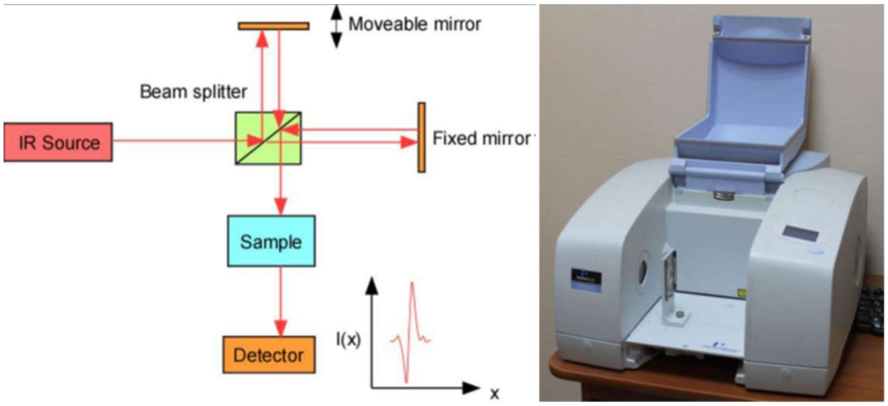


Figure. 4.8 depicts the operational principles of a Fourier transform infrared (FTIR) spectrophotometer and includes a photograph of the instrument.

4.3.4. Transmission Electron Microscopy (TEM)

A TEM is an electron microscopy device that achieves a highly magnified and detailed specimen image by focusing a beam of electrons on the specimen. The image of the specimen is produced with a magnification power that is more than 2 million times greater than that of the light microscope. This allows for convenient characterization of the image's morphological features, compositions, and crystallization information. In this method, electrons are generated by a heated tungsten filament in the electron cannon, which is then focused on the specimen by the condenser lenses (Figure 4.9). Upon reaching the specimen, the electrons are scattered, which focuses them on the magnetic lenses, resulting in a large, clear image. If the specimen passes through a fluorescent screen, it forms a polychromatic image. The electrons are dispersed more in a denser specimen, resulting in a darker image. This is because fewer electrons reach the screen for visualization, while thinner, more transparent specimens appear brighter.

The TEM operates on the same fundamental principles as the light microscope but employs electrons instead of light. The electrons possess a higher resolution capability due to their de Broglie wavelength, which is substantially smaller than light's, and their dual nature as both wave and particle. This allows the instrument user to analyze minute details, including a single column of atoms that is tens of thousands of times smaller than the smallest resolvable object in a light microscope.TEM is a significant analytical technique in various scientific disciplines, including physical, chemical, and biological sciences. The contrast in the TEM image at lower magnifications results from the material's thickness, composition, and absorption of electrons. The intensity of the image is modulated by complex wave interactions at higher magnifications, necessitating expert analysis of the observed images. The TEM can observe modulations in chemical identity, crystal orientation, electronic structure, and sample-induced electron phase shift in addition to the standard absorption-based imaging, through alternate modes of use.

The sample was examined by TEM analysis on a TECNAI 20 G2-Electron Microscope that was operated at an accelerating voltage of 200 kV in the current investigation (Fig. 4.9). The samples were prepared by mounting the dilute solution of the sample on a carbon-coated TEM grid and drying it under a table lamp for 5 hours. Subsequently, the samples were vacuum-dried overnight.

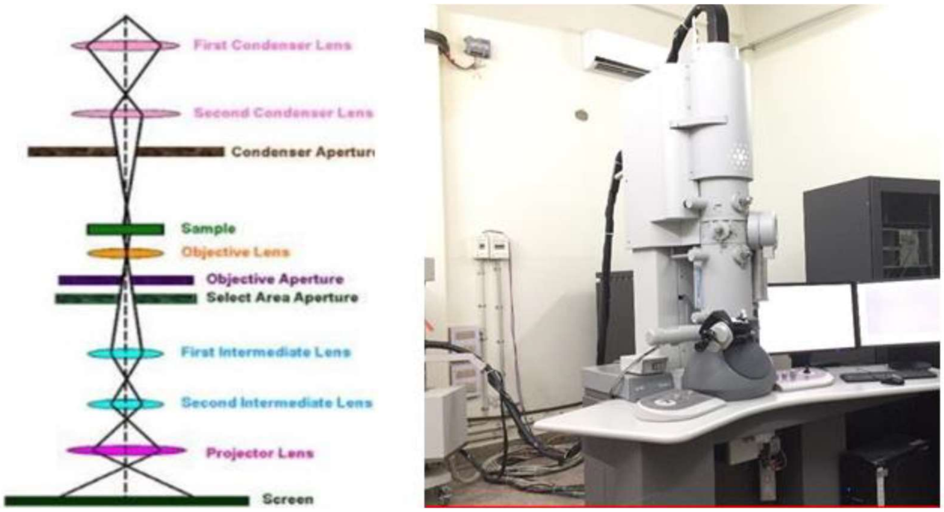


Figure. 4.9 Schematic representation of the principles of transmission electron microscopy (TEM) and a photograph of a TEM.

4.3.5. Analysis of the mechanical and physical properties.

The density of the composite scaffold was identified in order to analyze its sintering behavior. The current density of the samples was determined using the Archimedes method, which involves measuring the weight of the samples in air and using water as the liquid medium. The OHAUS AX324 precision balance is used to measure the weight of samples. Determine compressive strength. Compressive strength refers to the capacity of a material to withstand mechanical load when subjected to compression. The compressive strength of the composite scaffold was determined by testing cylindrical samples. The compression test of the cylindrical samples was conducted using a Universal Testing Machine (H10KL, Tinus Olsen, USA). The cylindrical samples underwent testing by subjecting them to a load, and the resulting results were collected from the Universal Testing Machine (UTM). Five samples were taken at each temperature, and their corresponding values were recorded.