Chapter 3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Substrates

Tectona grandis sawdust and organic fraction of municipal solid waste (OFMSW) were used as substrates for biogas production. The preparation methods of sawdust and OFMSW for biogas production are discussed in section 3.4 and 3.7, respectively.

3.1.2 Chemicals and reagents

The chemicals of analytical grade were procured from Sigma Aldrich, Merck, and Himedia brands.

Reagents for neutral detergent fiber analysis

1. Neutral detergent solution (1 L): Sodium borate decahydrate 6.81 g, disodium ethylene diamine tetraacetate 18.61 g, sodium lauryl sulfate 30 g, 2-ethoxyethanol 10 mL, disodium phosphate anhydrous 4.56 g, distilled water 1000 mL.
2. Sodium sulfite and acetone

Reagents for acid detergent fiber analysis

1. Acid detergent solution (1 L): Sulphuric acid (1 N) 1L, cetyltrimethylammonium bromide (CTAB) 20 g.
2. Acetone

Reagents for lignin estimation

1. Saturated potassium permanganate (1 L): distilled water 1 L, $KMnO_4$ 50 g, Ag_2SO_4 0.05 g.
2. Lignin buffer solution (1 L): ferric nitrate nonahydrate 6 g, silver nitrate 0.15 g, acetic acid glacial 500 mL, potassium acetate 5 g, tertiary butyl alcohol 400 mL, distilled water 100 mL

3. Combined permanganate solution: two parts of saturated potassium permanganate solution were diluted with one part of lignin buffer solution.
4. Demineralising buffer (1 L): Oxalic acid dihydrate 50 g, 95 % ethanol 700 mL, concentrated HCl (12 N) 50 mL, distilled water 250 mL.
5. Ethanol 80 %, acetone.

Reagents for sCOD estimation

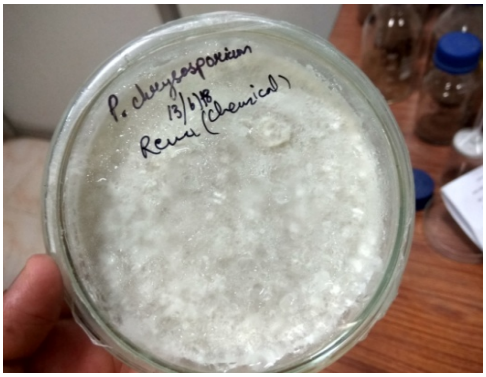
1. Reagent A (100 mL): $K_2Cr_2O_7$ 2.3 g, concentrated H_2SO_4 20 mL, $HgSO_4$ 3.34 g, distilled water 80 mL.
2. Reagent B (100 mL): Ag_2SO_4 1 g, concentrated H_2SO_4 100 mL.
3. Potassium hydrogen phthalate standard: KHP 0.425 g, distilled water 500 mL. 1 mg = 1 mg COD.

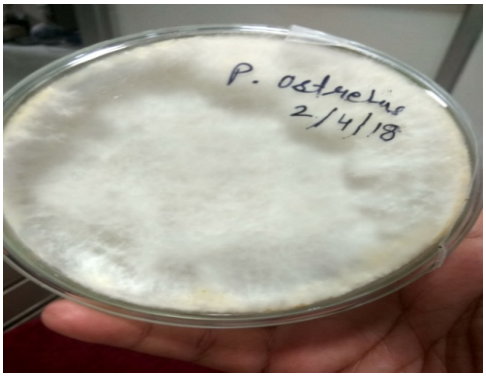
Reagents for glucose determination

1. Dinitrosalycyclic acid reagent (DNS reagent) (1 L): 127.5 g Rochelle salt, 4.4 DNS,1.25 N NaOH 294 mL, phenol 3.5 g, sodium bisulfite 3.5 g and distilled water 1 L.
2. Standard glucose solution (100 mL): glucose 0.5 g and distilled water 100 mL.

3.1.3 Microorganisms

Two fungal strains *Phanerochaete chrysosporium* and *Pleurotus ostreatus* were received from the National Collection of Industrial Microorganism (NCIM), Pune as slant culture. The fungal strains were revived in potato dextrose agar media with composition in 1 L: potato 200 g, dextrose 20 g, agar 15 g, and yeast extract 0.1 g. The fungal strains are depicted in Figure 3.1. The same media was used to maintain the fungus in viable condition by reviving it every 30 d.





(a) (b)

Figure 3.1 Fungal strains (a) *P. chrysosporium* (b) *P. ostreatus*

3.2 Analytical methods

3.2.1 Proximate analysis

Estimation of total solid (TS) in the substrate

The laboratory analytical protocol developed by National Renewable Energy Laboratory (NREL) was implemented for the estimation of total solid (Sluiter et al., 2008a). The sample was thoroughly mixed and 1 g sample was added into a previously oven-dried (105 ℃, overnight) crucible. The crucible was weighed before putting the sample. The crucible containing the sample was placed in a hot air oven (Khera instruments KI-181, New Delhi) at a temperature of 105 ± 1 ℃. The sample was dried to constant weight. Then it was cooled to room temperature in a desiccator. Finally, the weight of the crucible with the sample after drying was measured. The total solid and moisture in the substrate were calculated by using the following equations:

$$Total\ solid\ (\%) = \frac{Weight\ of\ crucible\ wit\quad sample\ after\ drying - Weig\quad of\ empty\ crucible}{Weigh\quad of\ sample\ as\ recieved} \times 100 \qquad (3.1)$$

$$Total\ moisture\ (\%) = 100 - total\ solid\ \% \qquad (3.2)$$

Estimation of ash in the substrate

Ash determination was carried out by the NREL procedure (Sluiter et al., 2008c). Crucible was ignited at 575±2 ℃ for 1 h in a muffle furnace (Khera instruments KI-180, New Delhi). Then it was placed in a desiccator for cooling to room temperature. The weight of the empty crucible was measured. Previously oven-dried (105±1 ℃) sample (1 g) was taken into the crucible. Crucible was placed in a muffle furnace and heated at 575 ℃ for 3 h. It was then placed in a desiccator for cooling to room temperature. Weight of crucible with sample after heating was measured and ash content was determined as follow:

$$Ash\ content\ (\%) = \frac{Weigh\ \ of\ crucible\ with\ sample\ after\ heating - Weight\ of\ empty\ crucible}{Weigh\ \ of\ oven\ dried\ sample} \times 100 \quad (3.3)$$

Estimation of volatile solid (VS) in the substrate

Volatile solid in the substrate (1g) was estimated by heating the sample at 550±2 ℃ by covering the crucible with a lid for 2 h (EPA Method 1684). Weight of empty crucible and with sample after heating was taken and the volatile solid was estimated as follow:

$$Volatile\ solid\ (\%) = \frac{Weight\ of\ initial\ sample - Weight\ of\ final\ sample}{Weight\ of\ initial\ sample} \times 100 \quad (3.4)$$

Fixed carbon was estimated by subtracting the sum of weight percentage of moisture, volatile solid and ash from 100.

3.2.2 Ultimate analysis

The measurement of carbon, nitrogen, and hydrogen was done by using an automatic CHNS analyser, Euro Vector EA, Italy. The instrument works on the principle of converting the elements into respective gases by combustion. After the combustion of

the sample the gases NO_x, CO_2, H_2O, and SO_2 are formed and get detected by a TCD detector.

3.2.3 Compositional analysis

Cellulose, hemicellulose, and lignin were determined by following the Van Soest procedure of fibre analysis (Soest and Wine, 1968). In this method, complete fibre analysis is carried out by estimating the neutral detergent fibre (NDF), acid detergent fibre (ADF) and dissolving lignin to separate lignin and cellulose.

Neutral detergent fibre (NDF) analysis

1 g of the sample was taken in a Kjeldahl flask. 0.5 g sodium sulfite was added followed by the addition of 100 mL of neutral detergent solution. Flask was refluxed for 1 h in presence of condenser. Filtration of the sample took place by using Gooch crucible (50 mL) and vacuum filtration assembly. The sample was washed with boiling water two times without losing the particles from the crucible. Then the sample was washed with 25 mL acetone. The crucible was put in an oven at 105 ℃ for overnight. Weight of empty and crucible with sample after drying was recorded. The NDF of the sample was found by the equation as given below:

$$NDF\ (\%) = \frac{(Weigh\ \ of\ initial\ sample - Weig\ \ \ of\ sample\ after\ drying)}{Weigh\ \ of\ initial\ sample} \times 100 \qquad (3.5)$$

Acid detergent fibre (ADF) analysis

1 g sample was added to 100 mL of acid detergent solution in a Kjeldahl flask. The flask was reflux for 1 h in the presence of condenser. The sample was filtered through previously weighed Gooch crucible by using vacuum filtration. It was washed two times with boiling water followed by acetone washing. The crucible containing the sample was dried at 105 ℃ in an oven for overnight. The weight of the crucible was measured after drying. The ADF of the sample was estimated as:

$$ADF\ (\%) = \frac{(Weight\ of\ initial\ sample - Weight\ of\ sample\ after\ drying}{Weigh\ \ of\ initial\ sample} \times 100 \quad (3.6)$$

The hemicelluloses content in the sample was calculated by the following equation:

$$Hemicellulose\ (\%) = NDF - ADF \quad (3.7)$$

ADF is the lignocellulose part of the cell wall. To separate the lignin and cellulose lignin is to be dissolved in lignin dissolving permanganate saturated solution.

Lignin/cellulose determination

The ADF was followed by lignin solubilisation to separate lignin and cellulose. 25 mL of combined permanganate solution was added to the ADF containing Gooch crucible. A short glass rod was placed inside the crucible to stir the material and break the lumps. Crucible was treated with this solution for 90 min. The solution was replaced by a fresh solution to maintain the purple colour. After 90 min the crucible was vacuum filtered and the demineralising buffer was added carefully to avoid the loss of particles from the crucible. This step was repeated until the fibre colour was changed from brown to white. Then, washing of fibre with 80 % ethanol was done for two times and then followed by acetone washing two times. The crucible containing fibre was kept at 105 °C overnight followed by weighing. The lignin and cellulose content in the sample were determined by the following equations:

$$Lignin\ (\%) = \frac{Weigh\ \ of\ ADF - Weight\ of\ final\ sample\ after\ drying}{Weigh\ \ of\ ADF} \times 100 \quad (3.8)$$

$$Celluose\ (\%) = ADF - Lignin \quad (3.9)$$

3.2.4 Calorific value estimation by bomb calorimeter

The calorific value was estimated by using bomb calorimeter (Rajdhani scientific, NSTTS Co., New Delhi, India) by ASTM method (ASTM D2015-96). Exactly, 1 g of the sample was measured and taken in a crucible followed by placing it inside the

calorimeter. A piece of firing wire was stretched straight across the electrode. A small length of thread was tied around the wire. The crucible was placed in such a way that loose ends of the thread were in contact with the material. The bomb was pressurised with oxygen at a pressure of 25 atm without displacing its original air content. After that, 2000 mL distilled water was poured into the calorimeter vessel and a bomb was placed in it. Ignition wire terminals were placed in two sockets of the electrode. The stirrer was adjusted and a thermometer was inserted. The stirring was started and the "Fire" button was pushed. The temperature variation was noted at each minute until no variation was observed. The calorific value was calculated by the following equation:

$$CV = \frac{W \times \Delta T - (CV_t + C_w)}{M} \quad (3.10)$$

Where CV is calorific value, W= 2248 cal, ΔT is a temperature difference, CV_t is the heating value of thread, CV_w is the heating value of wire, and M is mass of the sample.

3.2.5 Preparation of spore suspension and spore count

Four fully grown Petri plates were taken in laminar airflow. Then 3 mL of sterilised distilled water was added to the plates. The plates were slightly swirled and water containing spores were transferred to a sterilised centrifuge tube. The dilution of spores was done and spore count was taking place by using a hemocytometer.

Hemocytometer was carefully wiped by using 70 % ethanol. The shoulders of the hemocytometer were moistened and a coverslip was inserted to cover the area undercounting the chamber. A small portion of the sample was inserted into a hemocytometer through the edges of the coverslip. The hemocytometer was put under the fluorescence microscope (Olympus CKX53, UK) connected with a computer. The microscope was adjusted at 100 X magnification. The spores were counted in every 5 corners and the average spore value was calculated as:

$$Spores\ (spores/ml) = No\ of\ average\ spores\ \times 10^4 \quad (3.11)$$

3.2.6 sCOD estimation

sCOD estimation was carried out by using an automatic COD analyser (UNIPHOS, India). Standardisation of COD analyser was done to read the COD 1000 mg/L by using a blank and standard solution. 1 mL of sample was added to the COD tube. 0.6 mL of reagent A was added followed by 1.4 mL of reagent B addition. The tube was tightly capped and gently shook to mix the layers. The tube containing the sample was placed at 150 ℃ for 120 min. The tubes were cooled to room temperature after the digestion period was over. The COD was measured by placing the tube inside the analyser.

3.2.7 Glucose determination

Glucose in samples was measured by the DNS method given by Miller (Miller, 1959). A standard curve was generated from a stock solution of glucose having concentration of 5 mg/ mL. For glucose estimation in samples, a 1 mL sample was pipetted into a test tube. 3 mL DNS reagent was added to the sample and test tubes were incubated in a boiling water bath for 5 min. A blank containing distilled water and DNS was also run in parallel. It was then cooled and diluted to 100 ml. The absorbance was measured at 540 nm by spectrophotometer (ELICO SL 159).

3.2.8 Volatile fatty acids (VFA) determination

The titration method was used to estimate volatile fatty acids. 20 mL sample was put into a beaker and pH was noted. Then the sample was titrated with 0.1 N HCl to reach pH 4. The volume of HCl was noted. Then the sample was boiled for 3 min and cooled to room temperature. Then titration of the sample with 0.01 N NaOH was done to reach the pH 7. The volume of NaOH was recorded to reach pH from 4-7. The VFA was calculated by the following equation:

$$VFA\ \left(mg\frac{CH_3COOH}{L}\right) = 87.5 \times Volume\ of\ NaOH\ to\ reach\ pH\ 4-7 \qquad (3.12)$$

3.2.9 Phenolic content determination

Phenolic content was determined by using Singleton's Folin-ciocalteu reagent method (Singleton and Rossi, 1965). A standard curve was generated by using gallic acid stock solution. The 0.5 mL test sample was added to a test tube. 2.5 mL of 10 times diluted Folin ciocalteu reagent was added followed by addition of 2 ml of 7 % sodium carbonate solution. The test tubes were then incubated at 40 ℃ for 1 h. The phenolic content in terms of gallic acid (mg/L) was estimated by taking absorbance at 765 nm using UV-VIS spectrophotometer (ELICO SL 159).

3.2.10 Fourier transform infrared spectroscopy (FTIR)

KBr method was used to make the pellets for FTIR analysis. The sample was ground to achieve smaller particles. Then 1/8" micro spatula sample was added to 0.5 teaspoon of KBr and mixed thoroughly in a mortar pestle. It was then pressed in a press to form the pellets. The prepared pellets were placed in FTIR sample holder and the absorbance was read by Nicolet 5700 FT-IR spectrometer (USA) from 4000-400 cm^{-1} wavenumber.

3.2.11 X-ray diffraction analysis (XRD)

XRD analysis was done by using X-ray diffractometer Rigaku miniflex 600, Japan. The current and voltage applied were 15 mA and 40 kV, respectively. The XRD patterns were received by varying the 2 θ angle from 5-60 ° with variation at a speed of 5 ° per minute and step width of 0.02 °. The crystalline nature was studied through XRD graphs and crystallinity index (CI) was calculated using the equation given by Segal (Segal et al., 1959) as shown below:

$$CI\ (\%) = \left(\frac{I_{002} - I_{am}}{I_{002}}\right) \times 100 \qquad (3.13)$$

Where I_{002} and I_{am} indicated the intensity of the highest peak of the crystalline region, i.e 2 θ ≈ 22 ° and lowest intensity of amorphous region, 2 θ ≈ 18 °, respectively.

The mean crystallite size (d) of the phase was calculated from the line broadening of the most intense reflection using the Scherrer equation:

$$d = (0.9*\lambda_1)/ (\beta*\cos\Theta) \quad (3.14)$$

where d is the mean crystallite diameter, 0.9 is the Scherrer constant, λ_1 is the X-ray wavelength (1.54056 Å), β is the effective linewidth of the observed X-ray reflection, calculated by the expression $\beta^2 = B^2 - b^2$ (where B is the full width at half maximum (FWHM), b is the instrumental broadening) determined through the FWHM of the X-ray reflection at 2Θ of crystalline SiO_2.

3.2.12 Scanning electron microscope (SEM) analysis

Surface microscopic studies i.e. surface structure deterioration of substrate were carried out was confirmed by a scanning electron microscope, ZEISS EVO 18 RESEARCH, Germany after hydrolysis. The images were captured at a changing magnification of 200-5000 X with 10 kV voltage. The sample was finely ground before placing it into the SEM sample holder. The sample was coated with gold to prevent the charge build-up.

3.2.13 Gas volume quantification

The gas volume was determined by using the water displacement method connected to the anaerobic digester. The digester was shaken manually for 1 min before measuring the gas volume and the collected water was measured in a measuring cylinder as gas volume. The gas volume was measured each day at the same time.

3.2.14 Gas composition analysis

The gas composition was determined by a gas chromatograph (GC) Nucon 5765 equipped with a thermal conductivity detector. The N_2 was used as carrier gas at a flow rate of 30 ml/min. The temperature of the injector, detector, and oven was set at 393, 393 and 363 K, respectively. The separating column used was Porapak-Q, having

dimension 2 m × 0.25 mm. The 300 µL biogas was injected into the GC for measuring the composition of the gas. The different calibration curves for CO_2, CH_4, and water vapour were generated before measuring the biogas.

3.2.15 Potassium determination in manure

The sample was dried at 105 ℃ before potassium determination in manure. 2.5 g of sample was added to 25 ml of 1 N ammonium acetate. It was shaken at 150 rpm for 30 min, followed by filtration by Whatman filter paper no 1 and measurement of potassium was done by using flame photometer ESICO 1382, India. KCl solution (20-100 PPM) diluted in ammonium acetate was used as a standard solution for detection.

3.3 Framework of models used

3.3.1 Response surface methodology (RSM)

RSM is a statistical and mathematical tool used to optimise the process parameters for the maximum possible outcome. It applies to the processes that are dependent on some variables. The main RSM designs used by various researchers are central composite design (CCD), Box-Behnken design (BBD), 3 factorial and Doehlert. The interactive effect can find out effectively by using RSM that is advantageous over the manual optimisation of a process where the effect of a single influencing factor can be studied in one time. BBD is less expensive than CCD because the number of experiments is less in BBD in comparison to CCD. Analysis of variance (ANOVA) is used to examine the data fitting into the model.

3.3.2 First order model for anaerobic digestion

The model is based on the first order substrate kinetics. The variation of the substrate over time is given below.

$$\frac{dS}{dt} = -kS \tag{3.15}$$

$$S = S_0 e^{-kt} \tag{3.16}$$

Where S denotes the substrate concentration at the time "t", S_0 is initial substrate concentration, k is rate constant.

$$M = \alpha\ (S_0 - S) \tag{3.17}$$

$$M = M_m(1 - e^{-kt}) \tag{3.18}$$

Equation (3.16) and (3.17) are representing the kinetic equation for anaerobic digestion. Differentiating equation (3.17) with respect to time

$$\frac{dM}{dt} = M_m k e^{-kt} \tag{3.19}$$

$$ln(\frac{dM}{dt}) = ln\ M_m k - kt \tag{3.20}$$

Where k is disintegration rate constant, M is cumulative biogas production at any time (t), and M_m maximum biogas production. Logarithmic application results in a straight line equation. The equation of the straight line was used to calculate slope and intercept that gives the value of k and M_m, respectively.

3.3.3 Modified Gompertz model

The modified Gompertz model is based on the assumption that the substrate is present in the system throughout the process and there is the slow growth of microorganisms is present at the starting and end of the process. The model provides a sigmoid curve. The model also considers the lag phase. The equation of the modified Gompertz model of anaerobic digestion is given by (Kafle and Kim, 2013):

$$M = M_m \exp\{-\exp\left[\frac{R_m e}{M_m}(\lambda - t) + 1\right]\} \tag{3.21}$$

Where, M_m is maximum biogas produced, λ is lag phase period and R_m is the maximum biogas production rate observed at a particular day, e is 2.71, and t is time

3.4 Sawdust collection and characterisation

Tectona grandis (Sagwan) Sawdust was procured from a local sawmill of Varanasi, India. It was washed to detach the impurities such as dust, sand particles etc., ground and sieved. The particle size 60-35 (250-500 μm) mesh was stored in an airtight container for experimental work. The proximate, ultimate, compositional characteristics and calorific value were measured in sawdust.

3.5 Sawdust pretreatment

Sawdust was pretreated to reduce the lignin content present in it. The chemical, thermo-chemical and biological treatments were done to modify the structural characteristics of sawdust.

3.5.1 Selection of potential chemical reagent

The primary goal of pretreatment was to reduce the complexity of sawdust by altering the cell wall components of the substrate with the key goal of delignification. The literature review suggested that the chemical treatment with the reagents such as ethanol, H_2SO_4, HCl, aqueous ammonia, NaOH, $Ca(OH)_2$ carried out potential results after the treatment. Therefore, the sawdust was treated with the 2 % solution of ethanol/ H_2SO_4/ HCl/ aqueous ammonia/ NaOH or $Ca(OH)_2$. The 5 g of sawdust was added to the 50 mL solution of each above-mentioned chemical reagent. The flask was then incubated at 35 ℃ for 24 h. It was then filtered through Whatman filter paper no 1. The hydrolysate was stored at 4 ℃ for the estimation of sCOD and phenolic content. The sCOD and phenolic content were measured to evaluate the lignin solubilisation indirectly and hence the effectiveness of treatment. Based on the results, the chemical reagent was selected for further experiments.

3.5.2 NaOH treatment

The 5 g of sawdust was submerged into a 50 ml solution of NaOH prepared in the range of 2-12 %. The mixture was then placed in an incubator at 35 ℃ for 24 h. The mixture was filtered through a Whatman filter paper 1 after the completion of treatment. The separated liquid portion was stored at 4 ℃ for the quantification of sCOD, VFA, glucose, and phenolic content. The solid fraction was neutralised by the washing of distilled water. It was then dried at 105 ℃ overnight for studying compositional characteristics and also structural variation through FTIR, XRD, and SEM.

3.5.3 NaOH-microwave treatment

The NaOH treatment was followed by the microwave irradiation (Whirlpool MW-30 BC, USA) at 140 ℃ for 15 min. After the pretreatment, the mixture was subjected to filtration. The sCOD, VFA, glucose and phenolic content were measured in the hydrolysate and the solid fraction was undergone FTIR, XRD and SEM analysis.

3.5.4 NaOH-autoclave treatment

The NaOH treated samples were subjected to autoclave (Khera instruments vertical autoclave, KI-171) treatment at 121 ℃ and 15 psi for 15 min. After pretreatment time was over the separation of solid and liquid fractions were done and estimation of characteristics of hydrolysate and treated solid portion was done to measure the extent of pretreatment. The methodology of NaOH and combined treatment is given in Figure 3.2. NaOH-autoclave treatment was optimised for autoclaving time to improve the sawdust hydrolysis. The autoclaving time was varied in the range of 15-120 min. The parameters sCOD, phenolic content, cellulose, hemicellulose and lignin contents were estimated to find out the best treatment condition.

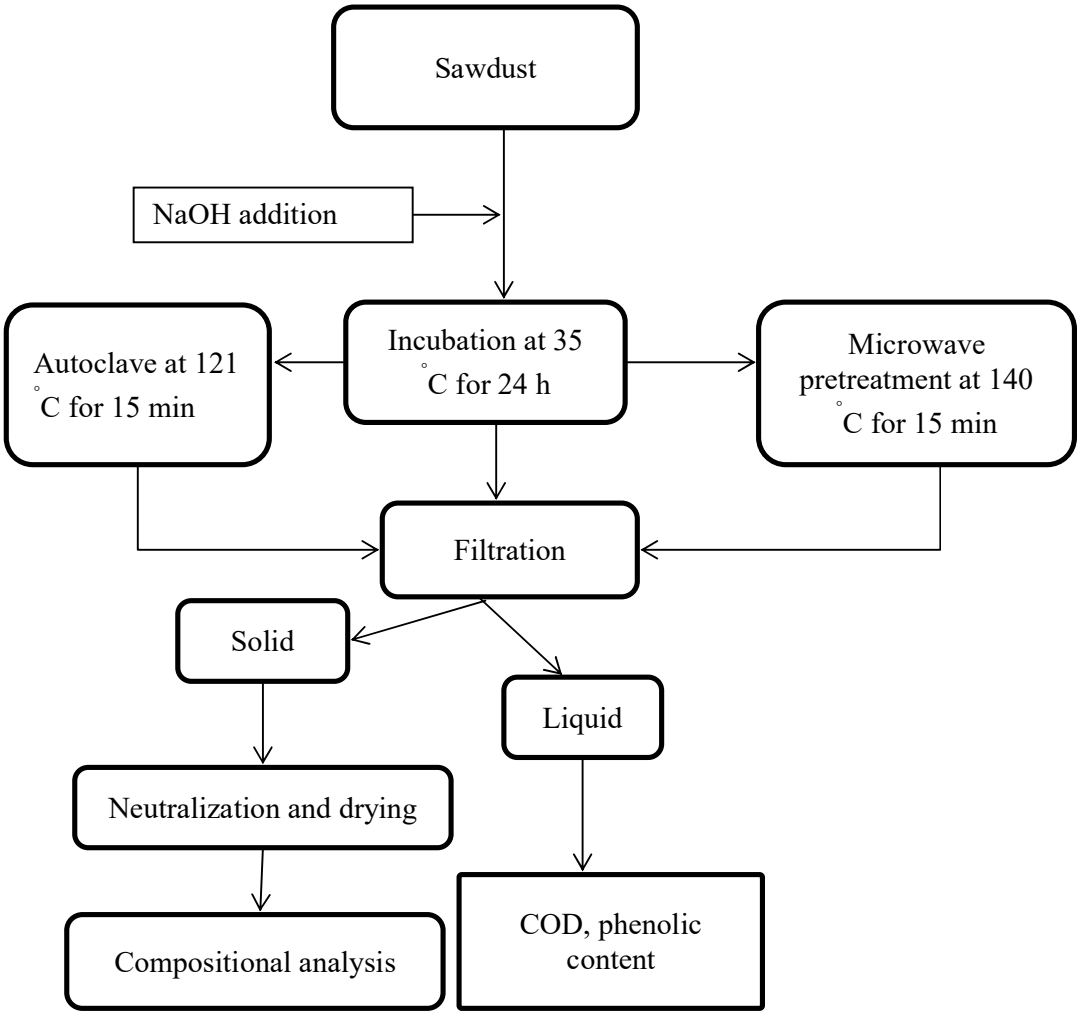


Figure 3.2 Methodology of NaOH and combined pretreatment of sawdust

3.5.5 Biological treatment

Two strains of white-rot fungi *P. chrysosporium* and *P. ostreatus* were employed for biological treatment. The spore suspension was prepared as discussed in section 3.2.5. The 5 g of sawdust was added to the conical flask, followed by the addition of 15 ml of water. The flasks were sterilised in an autoclave for 15 min at the 15 psi pressure and 121 ℃ temperature. The flask was inoculated with 2 ml of spore suspension (2.09×10^6 spores/ml) and incubated at 28 ± 1℃ for 10, 20 and 30 d. After the time of treatment

was over, the compositional parameters were analysed and surface morphological alterations were studied by XRD and SEM.

3.6 Anaerobic digestion of sawdust

Anaerobic digestion was done in a batch anaerobic digester. The digester was a four-necked round bottom flask. A pH probe and thermometer were inserted into the digester for pH and temperature observation as shown in Figure 3.3. N_2 was sparged for 5 min to maintain the anaerobic condition. The openings were tightly closed with rubber stoppers and vacuum grease. The system was kept in a waterbath to maintain the constant temperature throughout the process. The native and pretreated sawdust was digested at 30 °C. The inoculum was received from a biogas plant situated at Banaras Hindu University Campus, Varanasi, India. The plant was running on cow dung slurry. The inoculum was physicochemically characterised. The physicochemical characteristics of inoculum are given in Table 4.1. The substrate to the inoculum (S/I) ratio was maintained at 0.5 gVS of substrate/ gVS of inoculum. The reactor volume was 2 L with a working volume of 1.4 L. The retention period of digestion was 40 d. The rate constant and order of bioconversion of sawdust into biogas was also determined by the rate equation. The rate equation has been devised in terms of biogas yield against time as follow:

$$r_{bio} = k\, C_{bio}^{n} \tag{3.22}$$

$$\log\,(r_{bio}) = n log\, C_{bio} + \log k \tag{3.23}$$

Where r_{bio} is the rate of conversion of sawdust into biogas in NmL g^{-1}VS d^{-1}, C_{bio} is the biogas yield in NmL g^{-1}VS, k is rate constant, and n is order.

3.7 OFMSW collection and characterisation

Municipal solid waste (MSW) was collected from the dumping site of Varanasi. The 5 samples were collected every day up to 4 d from the different points of the same dumping site. The samples were collected immediately after fresh dumping. All the samples were mixed and the biodegradable part was separated manually. It was composed of food waste, vegetable and fruit waste, grass clippings, paper waste, cotton, textiles, etc.

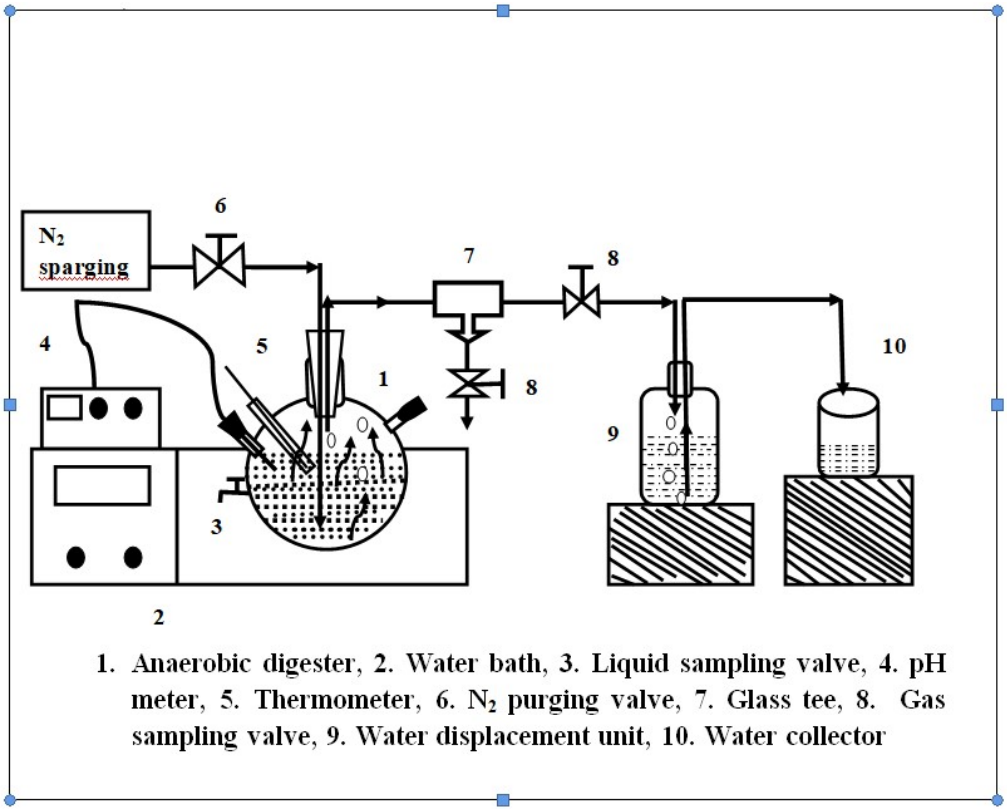


Figure 3.3 Schematic diagram of the anaerobic digestion system

The separated OFMSW was washed with water several times to separate the dirt from it. It was then ground in a kitchen mixture grinder to reduce the size. The raw OFMSW was characterised for TS, VS, moisture, C, N, H and O content. The sCOD, VFA and pH were also estimated for OFMSW slurry beside the above-mentioned parameters.

3.8 OFMSW pretreatment

3.8.1 Chemical treatment

NaOH emerged out to be the best pretreatment reagent in sawdust experiments. Therefore it was selected as a pretreatment reagent for OFMSW also. The chemical treatment was done by applying NaOH in the concentration of 3 g/L to the OFMSW (250 g sample). Then the OFMSW was chemically treated for 1 h. After the treatment time was over, the pretreated OFMSW was characterised for proximate and ultimate values, sCOD, VFA, FTIR, XRD, and SEM analysis.

3.8.2 Thermal treatment

The thermal solubilisation of OFMSW was carried out at 180 ℃ for 1 h. The actual set up for thermal and thermo-chemical treatment of OFMSW was shown in Figure A1 (Appendix). The same pretreatment parameters were analysed to compare the effects of each treatment.

3.8.3 Thermo-chemical treatment

Thermo-chemical treatment was done by adding the 3 g/L of NaOH into the OFMSW and heated at 180 ℃ for 1 h. The solubilised OFMSW was characterised for proximate, ultimate values, and the studies on structural variations were done through FTIR, XRD, and SEM analysis.

3.8.4 Biological pretreatment

Fungal strains *P. chrysosporium* and *P. ostreatus* were employed for biological treatment. The raw OFMSW was ground to form a homogenised slurry with the addition of some distilled water. The 100 mL of OFMSW was added to a 250 mL of a conical flask and sterilised in an autoclave for 15 min. The flask was inoculated with 3 mL of fungal spore suspension (spore concentration was 2.76×10^6 spores/ mL). The

flasks were incubated at 28 ±1 ℃ for 5, 10 and 15 d. Evaluation of VFA, sCOD, glucose and phenolic content was occurred to examine the variations after pretreatment.

3.9 Anaerobic digestion of OFMSW

The effect of each pretreatment on biogas production was also estimated. The OFMSW after chemical, thermal, thermo-chemical and biological treatment proceeded for anaerobic digestion. The digestion was carried out in a four-necked flask of 2 L capacity as shown in Fig 3.3. The photograph of actual anaerobic digestion set up for biogas production was shown in Figure A2 (a) of the Appendix. The substrate input and S/I ratio were 20 g VS and 0.5 g VS substrate/ g VS inoculum, respectively. The temperature and initial pH of the digester was 30 ℃ and 7, respectively for all the digestions from different pretreatment.

3.10 Optimisation of chemical treatment of OFMSW by RSM

The optimisation of chemical treatment was done by RSM. The mechanically liquidised OFMSW slurry was treated with NaOH at a varied concentration of 4, 20 and 36 g/L at temperature 35 ℃. The VFA and sCOD were determined to examine the effects of pretreatment on the solubilisation of OFMSW and set the NaOH range for RSM optimisation. The treatment was continued until there was no change observed in the VFA and sCOD value. The treated OFMSW was processed for anaerobic digestion.

Influencing factors and BBD design for chemical treatment of OFMSW

The effect of three influencing factors, the concentration of NaOH, time and temperature were evaluated on OFMSW solubilisation in terms of sCOD and VFA as the response. The concentration and time ranges were selected as 4-36 g/L and 24-72 h, respectively according to the hydrolysis results. The temperature range was selected as 20-40 ℃ per Indian climatic room temperature. The BBD design suggested 17 experiments in random with 5 central replicates. The model adequacy was determined

by analysis of variance (ANOVA) and significance of the model was checked by P-value, regression analysis, predicted R^2, adjacent $R^{2,}$ and C value. The 3-D plots were generated to study the interactive effect and optimise the influencing parameters for the better OFMSW solubilisation yield. The second-order polynomial equation to predict the model fitting used is as given below:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j + \varepsilon \quad (3.23)$$

Where, Y is denoting predicted response (sCOD, VFA), β_0 represents the constant regression coefficient, β_i, β_{ii} and β_{ij} are the coefficients for linear, quadratic and interaction parameter, respectively. X_i and X_j are the coded independent variables. 'ε' representing the residual associated with the experiments.

3.11Optimisation of thermo-chemical treatment of OFMSW by RSM

Three different sets of experiments were performed to set the ranges of influencing parameters. In one set of experiments, OFMSW slurry was treated with varying NaOH concentration in the range 1-7 g/L at a fixed temperature of 120 ℃ and time 1 h. In other sets, the temperature was varied from 90-200 ℃ at a fixed NaOH concentration of 5 g/L and time variation was done in the range of 15-120 min. The sCOD, VFA, and phenolic content were estimated and ranges of influencing factors were determined to execute the RSM model.

The temperature, NaOH concentration and time range were set 150-180 ℃, 3-6 g/L and 30-90 min according to the hydrolysis results (as shown in Figure 4.20, Section 4.9.1). A BBD design was generated by taking the three influencing factors. The sCOD, VFA, and phenolic content were the responses against the variables. The 17 experiments were suggested by BBD design. The statistical significance of the model was evaluated by ANOVA analysis. The 3-D plots were generated to study the interactive effect of the parameter.

3.12 Anaerobic digestion and kinetic modelling

Biogas production from chemical treatment was done at substrate input of 20 g VS and S/I ratio 0.5 (based on g VS) in 2 L flask. The experiment was performed for 4, 20, 36 g/L of NaOH and RSM optimised condition (18.4 g/L NaOH, 36.05 ℃ and 72 h). The biogas production from thermo-chemical treatment was also done in 2 L reactor at RSM optimised condition. The substrate input and S/I ratio were 20 g VS and 0.5 (based on g VS), respectively. The temperature and initial pH were kept at 30 ℃ and 7, respectively for both chemically/thermo-chemically treated samples. The first-order model and modified Gompertz model were used to study the biogas production pattern in both cases of anaerobic digestions. The models were compared using root mean square error (RMSE) (Kafle and Kim, 2013). The best fit model was considered if it exhibited less value of RMSE.

$$RMSE = \sqrt{\frac{1}{m}\sum_{j=1}^{m}\left(\frac{d_j}{Y_j}\right)^2} \tag{3.25}$$

Where 'm' denoted the no. of data pairs, Y_j is experimental biogas yield at j^{th} value and d_j is the deviation of the experimental and predicted value.

3.13 Co-digestion approaches

3.13.1 Suitable C/N ratio selection

The carbon/nitrogen (C/N) ratio was measured in different mixtures of OFMSW and activated sewage sludge (ASS) samples to determine the best C/N ratio for anaerobic digestion. The activated sewage sludge was procured from the sewage treatment plant (STP) Bhagwanpur, Varanasi, India. The sludge was physic-chemically characterised for proximate, ultimate and pH content, and it was mixed with OFMSW to achieve the OFMSW/ASS ratio as 4/1, 7/3, 3/2, 1/1, 2/3, 3/7 based on VS content of the material.

3.13.2 Optimisation of process parameters of co-digestion by RSM

The effect of three influencing factors, S/I ratio, pH, the temperature was evaluated on co-digestion and the best condition was achieved through RSM to maximise the biogas yield. The range of S/I ratio, pH and temperature were selected as 0.3-1.5, 6.5-7.5 and 30-60 ℃, respectively according to the literature study. The BBD design suggested 17 random experiments including 5 central replicates. All the experiments were performed twice and the results were incorporated in the BBD matrix. The ANOVA analysis was carried out to evaluate the adequacy of the model. The model significance was also determined by p-value, regression analysis, predicted R^2 and adjacent R^2.

3.13.3 Kinetic modelling of co-digestion

The experimental data from optimised co-digestion was compared using the first order and Gompertz model. The RMSE was also calculated to determine the best fit model.

3.14 Intermediate variation and end product characterisation

The variation in sCOD, VFA, pH and phenolic content in the hydrolysate of anaerobic digestion was noted during co-digestion at the optimised condition. The experiment was performed in a 2 L digester. The biogas was characterised for CO_2 and CH_4 content. All the characterisations were carried out in a 3 d gap up to the completion of the process. The slurry after completion of digestion was dried in a hot air oven at 105 ℃ and nitrogen and potassium content was estimated in it.