Chapter III – MATERIALS AND METHODOLOGY

This chapter describes the substrate composition and characteristics, experimental set-up, analytical procedure and analytical modelling techniques used for the present study.

3.1 Composition and Characteristics of OFMSW

Municipal Solid Waste Management (MSWM) has emerged as one of the global challenges. A developing nation like India has the greatest challenge of management of this increased MSW, due to population growth, economic growth, rapid urbanization, changes in lifestyle and other factors. The Ministry of Environment Forest and Climate Change (MoEFCC) and the Government of India have revised and framed the Solid Waste Management Rules, 2016 for effective waste management practice. The rules apply to every municipal authority responsible for the collection, segregation, storage, transportation, processing and disposal of MSW and support the objective of the Swachh Barat Mission (The Clean India program by the Government of India).

The MSW where OFMSW refers to the biodegradable portion of waste generated from household activities and commercial establishments. It is primarily composed of kitchen waste, food waste, yard waste, paper waste, fat-oil-grease (FOGs) etc having a high energy content (Aichinger et al., 2015; Bolzonella et al., 2006; A. Kumar & Samadder, 2017). MSW generated from cities comprises a maximum of organic fraction. OFMSW is known for its high organic matter as well as excellent biodegradability. Therefore, OFMSW can be treated using an anaerobic digestion process, an eco-friendly treatment technology that is useful to recover energy from waste. The composition of OFMSW is highly dependent on regional, seasonal, socio-economical and geographical conditions, availability, cultural usage of local products etc. (Iacovidou et al., 2012; Tyagi et al., 2018). MSW comprises a maximum bio-degradable waste of 42-53% which includes fruits & vegetable waste & food waste (Katiyar et al., n.d.; Sharholy et al., 2007; Sharma & Jain, 2019a; Thitame et al., 2010; Vinodbhai Mewada et al., 2020). The cities of India according to population size can generate an average of 41-52% of biodegradable waste (Saini et al., 2012). In the present study, wet OFMSW is considered (without plastic) including vegetable and fruit waste, food waste, yard waste and paper waste. Table 1 shows the composition of OFMSW adopted for the present study for lab-scale experimental work.

Table 1: Composition of OFMSW in the present study

OFMSW	% Wet mass
Vegetable and fruit waste: Banana peel, apple peel, orange peel, watermelon peel, cauliflower peel, chikoo and kiwi peel, cabbage peel, carrot peel, rotten vegetables & fruits (rotten potatoes, brinjal, bottle gourd, banana, tomato etc.	60-70
Food waste: cooked food (bread, chapati, khichadi, noodles, rice/biryani, cooked vegetables) etc.	20-30
Paper waste: packaging paper, newspaper pieces etc.	0.5-1
Yard waste: grass, leaves, flowers, soil etc.	2-5

Figure 1: OFMSW for lab-scale experiment

Lab-scale OFMSW is formed to reduce variance in its features (Figure 4). Different characteristics of biodegradable solid waste are observed from different research are shown in Table 2.

Table 2: Characteristics of OFMSW observed in different literature

Substrates	TS	VS	pH	TKN	Phosphate	COD	References
FW	21.2 ±2%	92.8±2%	4.7 ±0.7	-	-	-	(Dai et al., 2019)
Source Segregated OFMSW	289 ± 48.87 gm/kg	76% ± 7.8	-	11.4 ±7.3 gN/L	3.22 ± 1.20 gP/kgTS	-	(Bolzonella et al., 2006)
OFMSW	461.4 ±6.48 gm/kg	388.41± 6.41 gm/kg	6.84± 0.02	-	-	Soluble 94.87±1.86 gm/kg	(Dasgupta & Chandel, 2020)

Table 3 depicts the physicochemical characteristics of OFMSW, which is used for lab-scale experimental work

Table 3: Physico-chemical characteristics of OFMSW

Parameters	**OFMSW**
pH	5.8±0.4
%TS	18.17±2.14
%VS (of TS)	93.81±0.15
%Moisture content	81.82±0.86
COD (mg/gm)	265.6±16.93
TKN (mg/gm) (dry basis)	10.18±1.95
PhosphatePO_4^{-3} (mg/gm) (dry basis)	1.16±0.28
%TOC (dry basis)	50.59± 1.36

Various studies conducted on OFMSW reveal that VS ranges from 87% to 91%, while its pH remains between 3.9 to 4.2. Additionally, 10.5% to 12.5% of TS is found in OFMSW. When %TS >15, the anaerobic digestion process is considered to have high solids (Dai et al., 2013a).

Furthermore, different investigations of OFMSW have shown that total phosphorus ranges between 1.9 to 4.2 (g/kg TS) and the percentage of Total Organic Carbon (%TOC) ranges from 45.2 to 52.1. (Bouallagui et al., 2005)

3.2 Characteristics of bio-flocculated sludge from SST (post-UASB)

In recent years, treatment of domestic sewage with the UASB treatment technology has proven an attractive and appropriate option for developing countries due to lower operation and maintenance costs, lower sludge production and energy generation in the form of biogas. Unfortunately, sewage treated with UASB-based treatment technology could not meet Indian effluent discharge standards due to residual organic matter, nutrients and a significant count of pathogens (Vashi et al., 2019).To comply with effluent discharge norms set by the National River Conservation Directorate (NRCD) of the Indian MoEF, further treatment of UASB effluent is required due to some weak points of the UASB process (Sato et al., 2006). Efficient post-treatment processes adopted for treated sewage with UASB are extended aeration, MBBR, SBR etc. followed by SST which can meet discharge standards in terms of organic matter and nutrient removal to avoid environmental pollution. Sludge generated from the SST (post-UASB) process is rich in microbial activity so it is called bio-flocculated sludge. In the present study bio-flocculated sludge generated from SST is collected from 43MLD UASB-based sewage treatment plants with aeration treatment (ASP treatment) post-UASB. Table 4 provides the physicochemical characteristics of this bio-flocculated sludge which is rich in nutrients and the most projecting co-substrate for anaerobic co-digestion of OFMSW.

Table 4: Physico-chemical characteristics of bio-flocculated sludge

Parameters	**Bio-flocculated sludge**
pH	7.9±0.2
%TS	5.24 ± 1.18
%VS (of TS)	53.97±0.92
%Moisture content	94.76±0.6
COD (mg/gm)	52.22±4.03
TKN (mg/gm) (dry basis)	4.75±0.36

PhosphatePO_4^{-3} (mg/gm) (dry basis)	4.89±1.08
%TOC (dry basis)	28.52±1.52

Characteristics of WAS, sewage sludge, and dry sludge from different literature observed are given in Table 5.

Table 5: Characteristics of municipal sludge observed in different literature

Substrates	TS	VS	pH	TKN	Phosphate	COD	MC	References
Sewage sludge	12.5 ±1 %	6.5 ±1.5%	7.1± 0.2	-	-	40± 1.5 gm/L	87.5 ± 1.2%	Arelli et al., 2021
WAS	26 ± 3.78 gm/kg	69 ± 1.40%	-	4.9 ± 0.54 %TS	1.8 ± 0.27 %TS	-	-	Bolzonella et al., 2006
Dewatered Sludge	20.4 ±1.1%	56.7 ± 3.3 (VS/ TS%)	7.5 ± 0.2	-	-	-	-	Dai et al., 2013b

3.3 Characteristics of OFMSW & bio-flocculated sludge with different mixing ratios

The study further explains the synergistic effects associated with the co-digestion process. Different mix ratios of OFMSW & bio-flocculated sludge (50:50, 75:25, 90:10, 0:100 and 100:0) are taken for experimental work. The substrate is mixed based on the % wet mass. Changing in mixing ratio also improves the anaerobic co-digestion process due to changes in the physico-chemical characteristics of the substrate. Table 6 shows the different physico-chemical characteristics with varied mixing ratios of substrates which is used for batch experimental study.

Pre-treatment of OFMSW is required to improve the anaerobic co-digestion process. Pretreatment is given to the substrate in various literature such as mechanical, chemical or biological, biochemical, thermal, alkaline or hybrid methods (Dai et al., 2019**,** Rabii et al., 2021). The size of particles can be reduced to enhance the substrate utilization rate by the anaerobic microbes (Kim et al., 2000). Different pretreatment techniques are applied for anaerobic co-digestion for substrate food waste & poultry manure pretreated with autoclave, microwave and ultrasonication causing an increase in biogas yield 4.67, 6.43 and 10.12% respectively (Deepanraj et al., 2017a). The paraacetic acid oxidation treatment applied to sludge can effectively solubilize organic matter and increase biogas yield by 21% (Lise Apples, 2011). Apart from process efficiency, the factors that determine the success of the pretreatment methods are economical (cost reduction) and eco-friendly (reduce carbon footprints) (Tyagi et al., 2018). Cutting and grinding is the simplest and most economically viable pretreatment method for the present study. The OFMSW is ground in a size of 2 mm ~ 5 mm. Grinding has allowed for an increase in the surface area that is available for microbes. Figure **5** describes the substrate preparation for anaerobic co-digestion in the study.

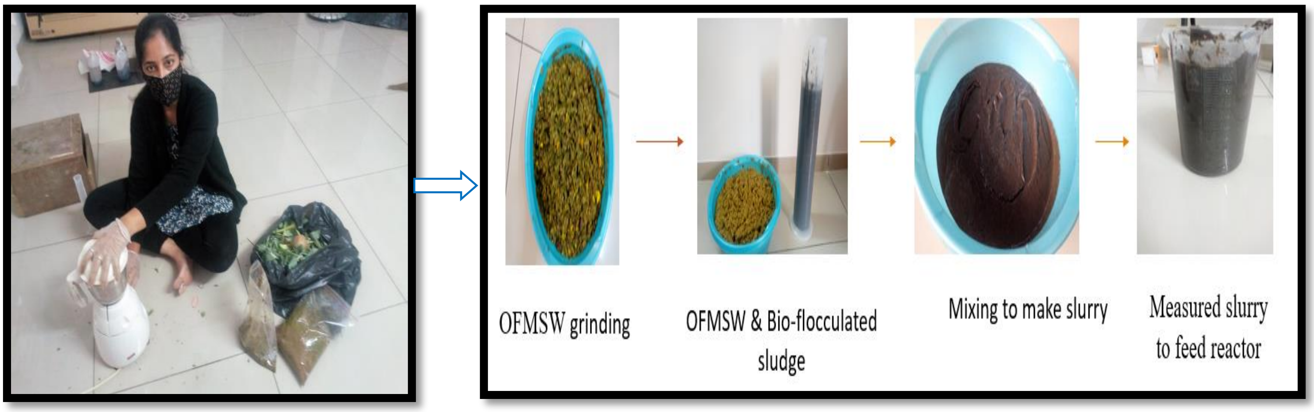

Figure 2: Pretreatment of OFMSW and substrate preparation

Table 6: Physico-chemical characteristics of the co-digested substrate with varied mixing ratio

Substrates→ Parameters↓	**50:50**	**75:25**	**90:10**	**0:100**	**100:0**
pH	6.37±0.2	6.16±0.1	6±0.1	8.08±0.1	5.84±0.2
%TS	6.96±0.27	15.26±0.44	18.5±0.38	5.05±0.20	19.04±0.26
%VS (of TS)	77.95±1.83	91.25±0.26	86.2±1.58	56.31±0.34	93.9±0.04
%Moisture content	92.36±0.21	84.16±0.18	81.12±0.11	94.96±0.06	80.85±0.28
COD (mg/gm)	168.96±2.28	191.8±0.14	185.6±3.7	52.24±3.07	256.38±4.52
TKN (mg/gm) (dry basis)	5.23±0.62	7.28±1.26	6.86±1.84	4.34±0.28	12.88±1.14
PhosphatePO_4^- (mg/gm) (dry basis)	3.26±0.24	1.01±0.16	1.45±0.12	5.74±0.06	1.25±1.06
%TOC (dry basis)	45.21±1.12	49.86±1.82	47.10±2.44	30.77±0.12	51.31±0.028

3.4 Fabrication of anaerobic reactor

Anaerobic reactor fabricated with an acrylic sheet of size 15cm*15cm*50cm ht. of cuboid shape. The reactor is installed with a 12 V DC motor and stainless-steel paddle for mixing the substrate. Sampling ports are provided at a distance of 10cm from the bottom with valves of 1/4 inch in the reactor for sampling the substrate during experimental work. One biogas outlet port is also provided to collect the biogas generated during the anaerobic process. The model of an anaerobic reactor is shown the Figure 6 (A). To make the reactor airtight it is sealed with nuts and bolts and a rubber gasket is also provided. During experimental work anaerobic reactor is required mesophilic conditions for the process. To maintain the mesophilic condition during temperature drop, the reactor is provided with a water jacketing system. The reactor is modified as shown in Figure 6 (B) provided water jacketing. To maintain the inside temperature of 30-35℃, the temperature of the water jacketing is maintained with a heating rod.

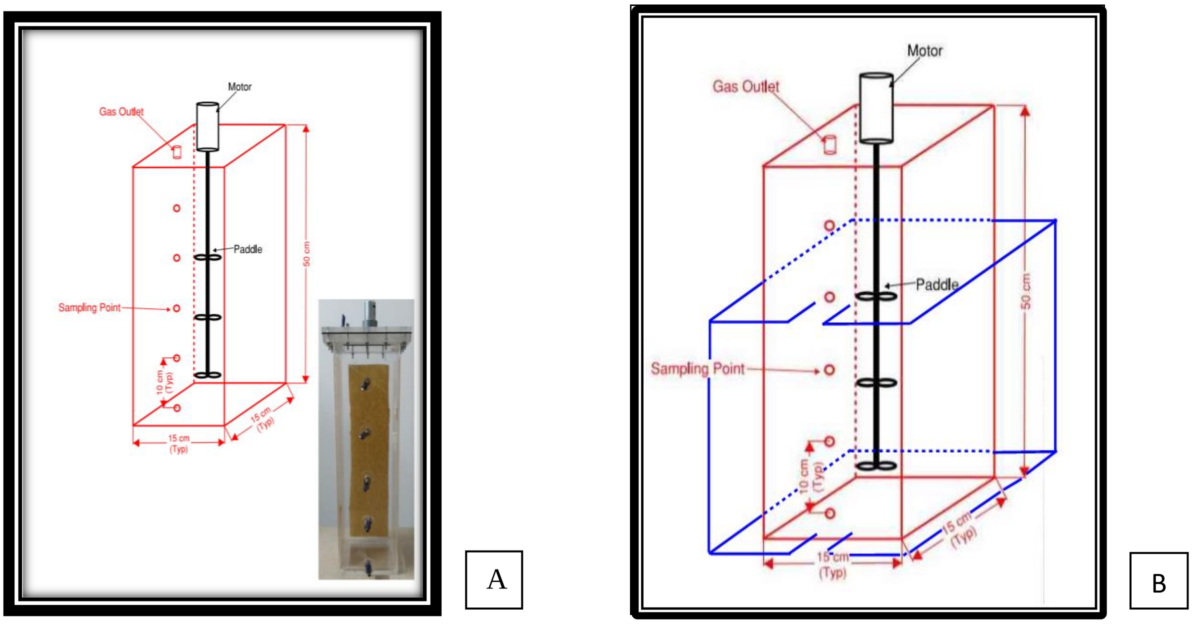

Figure 3: Fabrication of lab-scale anaerobic reactor

3.5 Experimental setup for anaerobic co-digestion process

An anaerobic reactor of 10 L volume is made of an acrylic sheet. The substrate is fed on a wet mass basis and each batch reactor is fed with a 7 kg feed. A mixer with paddles is used for complete mixing (intermediate mixing) of the substrate at low speed using a 12V DC motor. To maintain the mesophilic condition (30-35°C) inside the reactor, the water jacketing system is provided with a heating rod. This system effectively controls the inside temperature of an anaerobic reactor and provides optimal temperature conditions for the anaerobic digestion process. The biogas produced during the batch study is quantified using water displacement (Figure 7). The biogas is routed through the solution of NaOH which can absorb CO_2 from the biogas. The sample is collected from the sampling port at intervals of 24 hrs for the analysis. All the samples are analysed on a triplicate basis.

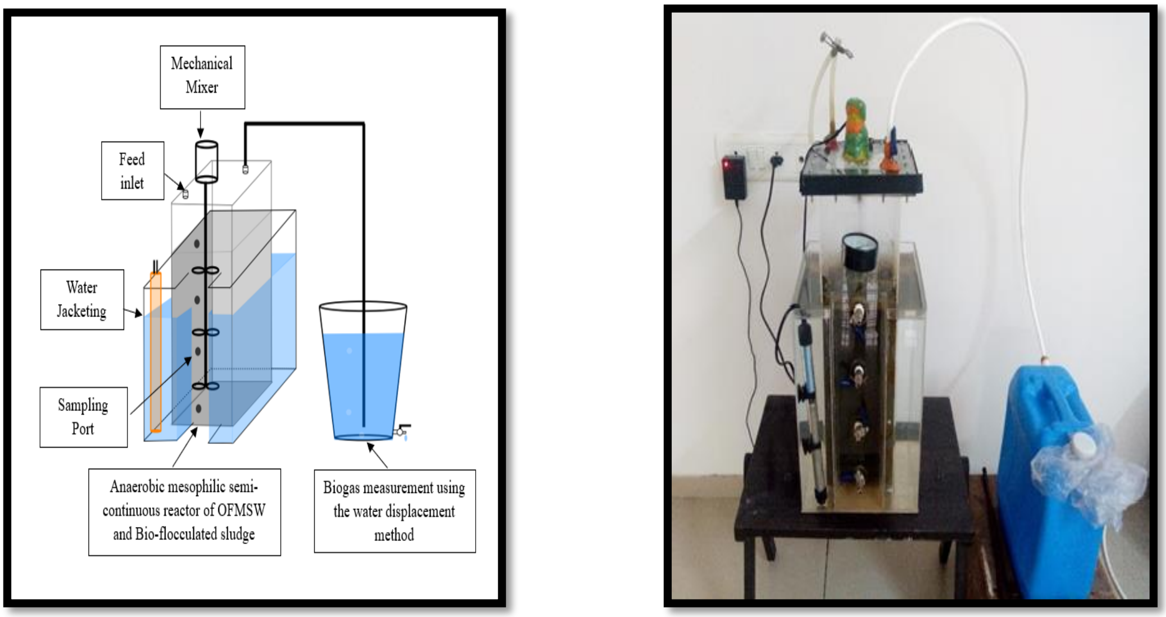

Figure 4: Schematic diagram and the lab-scale experimental setup

3.6 Methods of sample analysis

To determine the initial and final characteristics of the feedstock, the samples are prepared for analysis according to the procedures prescribed in APHA, Standard Methods for Examination of Water and Wastewater, 2017.

3.6.1 Analysis of Total Solids (%TS) and Volatile Solids (%VS)

TS and VS are measured regularly as per 2540-G (APHA,2017). The % TS and % are determined by drying the substrate at 103°C- 105°C in an oven for 24 hours and then further incinerating the substrate obtained from the oven, at 550°C in a muffle furnace for around 120 minutes. Volatile Solids are expressed as a percentage of Total Solids. Analytical Balance (model – AP225WD, Make – Shimadzu) was used with minimum displace of 0.1mg/0.001mg, Hot Air Oven (Model –Digital (SS) 0℃ to 250̊ C, Make Janki Impex), Digital Muffle Furnace (Model- Digital Temperature Controller, Make- Janki)

3.6.2 Analysis of Chemical Oxygen Demand (COD)

The measure of COD is carried out using the closed reflux method during the analysis of an anaerobic sample (5220 – C) (APHA,2017). To prepare a sample for COD analysis, 1 gram of effluent sample is acidified with 2 ml of concentrated H_2SO_4. The volume of the sample is made up of 10 ml with distilled water. The digested sample is utilized to measure the COD of the sample. After the addition of concentrated H_2SO_4 & $K_2Cr_2O_7$, the sample is digested for 2 hrs at 150℃ and then left at room temperature. Afterwards, it is titrated with Ferrous

Ammonium Sulphate (FAS) and COD is measured in mg/L. The sample used for analysis is 10ml which is equivalent to 1gm of the wet sample therefore COD is measured in mg/gm.

3.6.3 Analysis of pH

pH meter (ANALAB Digital pH meter – pHCal) is used to measure the pH of the sample from the reactor. The sample is centrifuged with Clinical Centrifuge, model 858/8, REMI electricals, @3500 rpm-10 minutes and supernatant is utilized to measure the pH of the actual sample.

3.6.4 Analysis of VFA/Alkalinity Ratio

The volatile Fatty acids to Alkalinity ratio is measured with Kapp's method (Mota et al., 2015). The anaerobic sample is centrifuged and the supernatant is taken for analysis. 20 to 30ml of centrifuge sample is taken for analysis and measured initial pH. Then titration of the sample is carried out with 0.1N H_2SO_4 till it is reached at pH 5 and the volume of titrant is recorded. Now slow and continue the addition of acid until pH reaches 4.3 and the measured volume added titrant A2 is recorded. The later pH 4 is achieved by repeating the same step and the volume of titrant A3 is recorded. The results are calculated by applying equations (1) & (2).

Alkalinity = A *N*1000/sample volume Equation 1

VFA= 131340*N*B/sample volume – 3.08*Alk-10.9 Equation 2

where,

Alk= Alkalinity (mmol/L)

A= 0.1N H_2SO_4 titrant used from initial pH to pH 4.3 (A=A1+A2(ml))

N= Normality (mmol/l)

SV= Sample volume

VFA= Volatile Fatty Acids (mg/L acetic acid equivalents)

B= Sulphuric acid to titrate sample from pH 5 to pH 4 (ml)

Alk= alkalinity (mmol/L),

Alklinity (mmol/L) *100 will convert in to mg/L

3.6.5 Total Kjeldahl Nitrogen (TKN) & Total Phosphate

Total Kjeldahl Nitrogen is determined with the Macro Kjeldahl method through digestion and followed by distillation and Total Phosphate with the Stannous Chloride method (4500 N_{org}-B, 4500P-D) (APHA,2017), respectively. 1gm of dried sample pre-digested in 5:1 HNO_3:H_2SO_4 solution for 2 hrs at 300℃ and absorbance of the sample is measured at 690nm using UV Spectrophotometer (model-UV-1800, make –Shimadzu). The calibration curve for phosphate is shown in Figure 8.

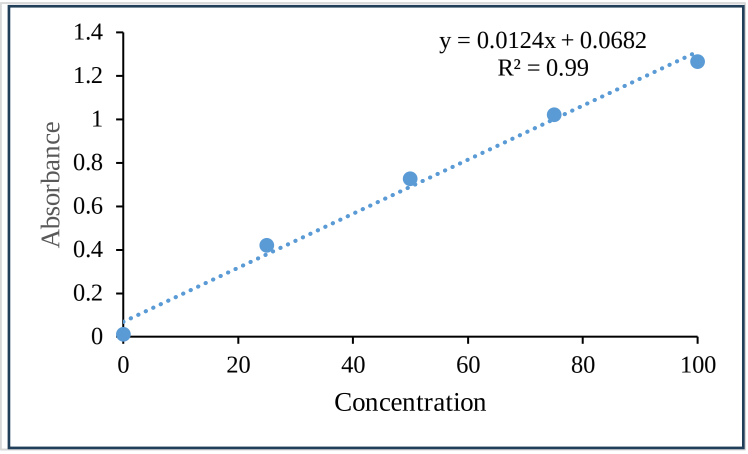

Figure 5: Calibration plot of phosphate

3.6.6 Microscopic Analysis

The sample retrieved from the methanogenesis phase is used to analyse microbial presence in an anaerobic co-digestion process. The whole sample and supernatant are taken for microscopic analysis under microscope OLYMPUS BX53. To get the microscopic image gram staining protocol was used (Gram Stain Protocols, 2019). The presence of gram-negative microorganisms in the samples shows pink or red colour under the microscope. The supernatant of the sample is also checked with the same procedure. To analyse the detailed image of the microbes, present in the sample the supernatant of the sample is analysed under Scanning Electron Microscope (SEM)- JEOL JSM-6380LV. The sample is spread over a 1cm*1cm cover sheet in a clean environment. The sample is observed under the SEM unit to identify the presence of microorganisms.

3.7 Analytical modelling techniques

3.7.1 Kinetic modelling for biogas in batch & semi-continuous flow anaerobic reactor

Three popular kinetic models have been chosen for the modelling of biogas produced during lab-scale experimental, batch and semi-continuous flow anaerobic reactor study.

3.7.1.1 First-Order kinetic model

First-order kinetic model:

$$M = P_m \times [1 - \exp(-kt)] \quad \text{Equation 3}$$

Equation 5 describes the methane yield over time according to a First-Order kinetic model.

In the First-Order kinetics for the hydrolysis of organic matter, the cumulative biogas production can be described with M-the amount of biogas production up to substrate availability up to time t and P_m is the initial amount of substance at the time t_0 when the process gets started. Constant k characterizes the speed at which substrate is consumed to generate biogas. $1 - \exp(-kt)$ term represents the fraction of the substrate that is produced up to time t. So, putting all together First-Order kinetic model represents the biogas at a rate proportional to the difference between the Initial amount P_m and the current amount M with a rate constant k which determines the speed of the process.

3.7.1.2 Modified Gompertz model

The BMP test shows that the cumulative methane yield data are well-fitted to the Modified Gompertz equation (Zwietering et al., 1990). Cumulative methane yield as well as the duration of the lag phase is also an important factor to determine the efficiency of the anaerobic co-digestion process.

Modified Gompertz Model: $M = P_m \times \exp -\{\exp \frac{[Rm \times e(\lambda - t)+1]}{P_m}\}$ Equation 4

The cumulative biogas production for time t can be achieved with a maximum achievable biogas yield, maximum biogas production rate and the required time duration where the maximum biogas yield rate can be achieved. The sigmoidal growth and saturation effects are in exponential terms providing insights into the dynamics of methane production in biogas systems.

3.7.1.3 Logistic Function model

Logistic Function Model: $M = \frac{P_m}{1+\exp 4xR_m(\lambda - t)P_m}$ Equation 5

The Logistic Function model describes the cumulative biogas production over time (t) with a maximum potential of biogas production at the maximum biogas production rate and time required to reach that production rate. It also captures sigmoidal growth patterns and saturation effects for biogas production. Multiplication of 4 in the exponent gives the steepness to the sigmoidal curve.

M is the biogas yield (L/kg VS_{added}) concerning time t (days), P_m is the maximum biogas potential of the substrate (L/kg VS_{added}), k is the hydrolysis rate constant (1/day), t is the time (day), R_m is the maximum biogas production rate (L/kg VS_{added}*d), λ is the lag phase time (days), e is Euler's function = 2.7183.

3.7.2 Kinetic modelling for substrate removal efficiency

Kinetic models help quantify how fast organic matter is degraded in the anaerobic digester which helps to select the most suitable feedstock and anaerobic digester performance(Shahzad et al., 2022). A kinetic model is a mathematical representation of the relationship between COD concentration and HRT for the estimation of degradation rates (Nkeiruka Nweke & Nwabanne, 2021). The rate at which COD or VS is removed directly impacts the rate of biogas production and kinetic models help to estimate and optimize the rate of biogas production by correlating with substrate removal rate. Various mathematical equations have been developed and effectively employed to forecast and explain the functioning of anaerobic reactors. The present study aims to assess the suitability of different kinetic models to connect organic matter concentrations at the inlet and outlet and to compare the precision of effluent COD concentration by applying three kinetic models (i) Modified Stover-Kincannon (ii) Grau's Second-Order and (iii) First-Order kinetic model on lab scale operated semi-continuous flow anaerobic reactor for anaerobic co-digestion of OFMSW and bio-flocculated sludge.

3.7.2.1 Modified Stover-Kincannon model

The Stover-Kincannon model was initially applied (1982) for the Rotating biological contractors. Afterwards, the equation is further modified for the application of another reactor also and is known as the Modified Stover Kincannon equation. There is a definite relationship between HRT and organic concentration with substrate rate and efficiency according to Kincannon and Stover(Nor Faekah et al., 2020).

The Modified Stover-Kincannon equation in terms of outlet substrate concentration can be written as equation 8.

$$Se = \mathrm{Si} - \frac{\mathrm{Umax} * \mathrm{Si}}{\mathrm{KB} + (\mathrm{Q} * \frac{\mathrm{Si}}{\mathrm{V}})} \qquad \text{Equation 6}$$

S_i= inlet substrate concentration (g/L); S_e = outlet substrate concentration (g/L); U_{max}= maximum removal rate constant (gm/L*day); K_B = Saturation constant (gm/L*d); Q = flow rate (L/d); V = Volume of the reactor (L)

The intercept and slope of the line show the value of kinetic constant U_{max} and K_B, respectively.

3.7.2.2 Grau's Second-Order kinetic model

Grau's Second-Order equation (9) obtained is given as below

$$\frac{HRT}{E} = \mathrm{a} + \mathrm{b} * \mathrm{HRT} \qquad \text{Equation 7}$$

Where, Substrate removal efficiency (E) = Si-Se/Si

The intercept and slope of the plot of HRT vs HRT/ $COD_{removal}$**,** determine the value of kinetic constants a and b.

Grau's Second-Order kinetic constant, specifically the parameters 'a' and 'b,' is a valuable tool for understanding and optimizing anaerobic co-digestion processes in terms of COD (Chemical Oxygen Demand) removal. It helps to identify the ideal range of substrate concentrations to maintain efficient COD removal without reaching inhibitory levels.

3.7.2.3 First-Order kinetic model for substrate removal

The kinetic model is used to explain the substrate removal rate of organic matter, in the anaerobic digester for concentration of effluent under pseudo steady state condition (Ahmadi et al., 2019b) can be written as equation (8)

$$\frac{Si - Se}{HRT} = \mathrm{kr} * \mathrm{Se} \qquad \text{Equation 8}$$

where S_i and S_e are the influent and effluent substrate concentrations (g/l), respectively; kr is the First-Order substrate removal rate constant (1/d); Hydraulic Retention Time (HRT) in days

The value of kr can be calculated by plotting Se versus [(Si – Se)/HRT].

The First-Order kinetic constant provides insights into how operational changes such as HRT and OLR affect the rate of COD removal and help to maximize biogas production.

3.8 Development of prediction model using Artificial Neural Network (ANN)

The human brain is a complex structure with a closely linked network of basic processing units, or neurons. Artificial Neural Network or Neural Network are the simplified representation of the organic nervous system. The mathematical relationships between variables to discover the link between a set of input variables and output variables with a wide range of operational conditions, a computer learning system ANN is used (Ramachandran et al., 2019). When a group of nodes or neurons are connected by synaptic connections, a neural network is created. The ANN consists of three layers: an input layer, a hidden layer, and an output layer. Input variables (%TS, OLR (gmVS/L/d), pH, HRT, VFA/Alkalinity ratio) and output variables (%$VS_{removal}$ and Methane yield (L/kg$VS_{removed}$) are included to develop the prediction model.

3.8.1 Development of Feed Forward Neural Network prediction model using fitting application

ANN study is conducted with MATLAB 2021a to implement the Feed Forward training algorithm with the curve fitting application (fitnet). The prediction model is developed with three training algorithms: Levenberg-Marquardt (trainlm), Bayesian Regulation (trainer) and Scale Conjugated Gradient (trainscg) along with the tan sigmoid transfer functions (Figure 9)

Data normalization is the initial step for any data analysis work. There are several normalization techniques but here in this study minimum-maximum method is applied. Input and output variables need to be correctly normalized to get reliable results. The minimum and maximum values of each variable are used to scale all data to the range [0,1] using Equation 9.

$$Xn = \frac{X - \mathrm{Xmin}}{\mathrm{Xmax} - \mathrm{Xmin}} \qquad \text{Equation 9}$$

X = experimental data

x_n = normalised value of the experimental data

x_{min} = minimum value of experimental data

x_{max} = maximum value of experimental data

Predicted output de-normalized using equation 10

$$Xn*[(X_{max} - X_{min})] + X_{min} = X \qquad \text{Equation 10}$$

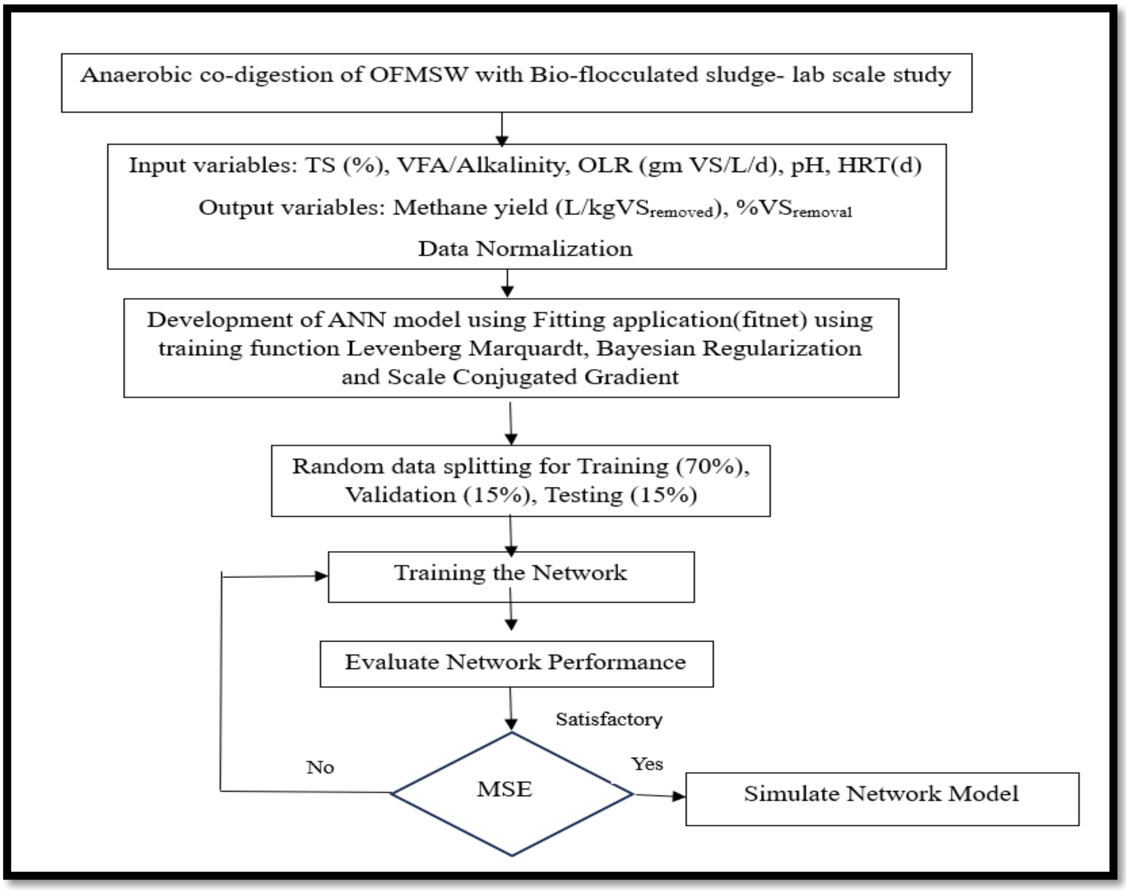

Figure 6: Feed Forward Neural Network (FFNN) flow diagram to develop a prediction model

3.8.2 Relative Importance of Input Variable (RI)

ANN models are typically utilised, to determine the association between input and output variables, ANN Connection weight method, Garson's algorithm and other various techniques are employed to evaluate the significance of variables like incomplete derivative, input disturbance, sensitivity evaluation, forward enhanced sequential selection and addition I, and Stepwise selection improved II (OLDEN, 2004). It demonstrates how input and output parameters are interdependent. The Connection Weight Approach is considered the best method used in this investigation.

The weight of the connection from input neurons to hidden neurons is added to the weight of the connection from hidden neurons to output neurons for all input parameters in the connection weight technique. The relevance of the parameters associated with the input neuron is inversely correlated with the total connection weights. The equation (11) is used to determine the input parameters' relative significance (i):

$$\mathrm{RI(i)} = \sum_{N=1}^{n} (CW(ih) \; * \; CW(ho)) \qquad \text{Equation 11}$$

RI(i) is the relative importance of the input parameters, CW(ih) is the connection weight from the input neuron to the hidden neuron, CW(ho) is the connection weight from the hidden neuron to the output neuron, n is the number of neurons in the hidden layer.

3.8.3 Development of prediction model using ANN-PSO

In recent years advanced computational tools have played a pivotal role in addressing this complexity. Artificial Neural Networks coupled with Particle Swarm Optimization (ANN-PSO) have emerged as powerful predictive modelling techniques for the anaerobic co-digestion process. ANN combined with the optimization power of PSO enables the development of accurate and efficient predictive models. The well-known optimisation technique PSO is influenced by the routine interactions of flocks of birds. James Kennedy and Russell C. Eberhart devised the Particle Swarm Optimisation (PSO) algorithm as a technique for the optimisation of continuous non-linear functions. It draws inspiration from observations of social and group activities, as well as flocks of birds and typical human behaviours (Antonio et al., 2011). The convergence rate of PSO is quite quick compared to other optimisation algorithms like the Ant and Bee method, Genetic Algorithm, Moth-Flame Optimisation Algorithm, and Cuckoo Search Firefly Algorithm, which speeds up its applicability in several study fields Each bird is referred to as a "Particle" and its floc is referred to as a "Population" in technical terminology (S. Kumar et al., 2013). The movement of all particles across a larger region in quest of food serves as their goal function (and also serves as my function). This particle's mobility is dictated by one's own or nearby neighbours' experiences. The initial population is the collection of all particle positions. Following the generation of random velocity, the objective value is assessed using the objective function. Personal best ("pbest") and Global best ("gbest") refers to the initial condition and position that correspond to the optimal value. Architecturally, this optimisation tool focuses on the population-based approach, in which the system introduces a population of random particles while simultaneously applying an algorithm to the population (swarm size) with a certain number of iterations and a certain maximum run to meet the fitness function (Alam et al.,2016). Update the local best and global best position that contributes to the achievement of the lowest objective function, together with the updated weight and bias.

3.8.4 Development of prediction model using ANFIS

Adaptive Neuro-Fuzzy Inference System (ANFIS) combines fuzzy logic and neural network approaches to create a hybrid intelligent model with the advantages of both methods. The input-output data collected from the lab-scale experiment of the anaerobic co-digestion process are used to develop the ANFIS model. %TS, OLR, pH, VFA/Alk ratio, and HRT are considered input variables for methane yield as output variables. Data normalization is applied for the performance of the ANFIS model. The ANFIS model is developed with the ANFIS toolbox on MATLAB. The first layer acquires (Sada & Ikpeseni, 2021)the input parameters and introduces them to the ANFIS model. ANFIS model consists of two main layers (i) fuzzy layer and (ii) adaptive layer where the fuzzy layer is used to apply membership function to input variables and the adaptive layer tunes the parameters of the fuzzy layer using a learning algorithm. Data is split into training and testing data and using a hybrid optimization algorithm ANFIS model is trained. The output layer is trained with a linear membership function. The goal of error is kept at zero to reduce the error between output data and the target variable. The network is trained with four membership functions. To select the type of membership function, training of the network is done with triangular-shaped, generalized bell-shaped, trapezoidal-shaped, and Gaussian membership functions. The training function adjusted the parameters of the membership function and rules of the ANFIS model. ANFIS model is evaluated with a testing data set with the lowest MAPE. A trained and tested ANFIS model is used for the prediction of methane yield under various conditions of the anaerobic co-digestion process (Figure 10).

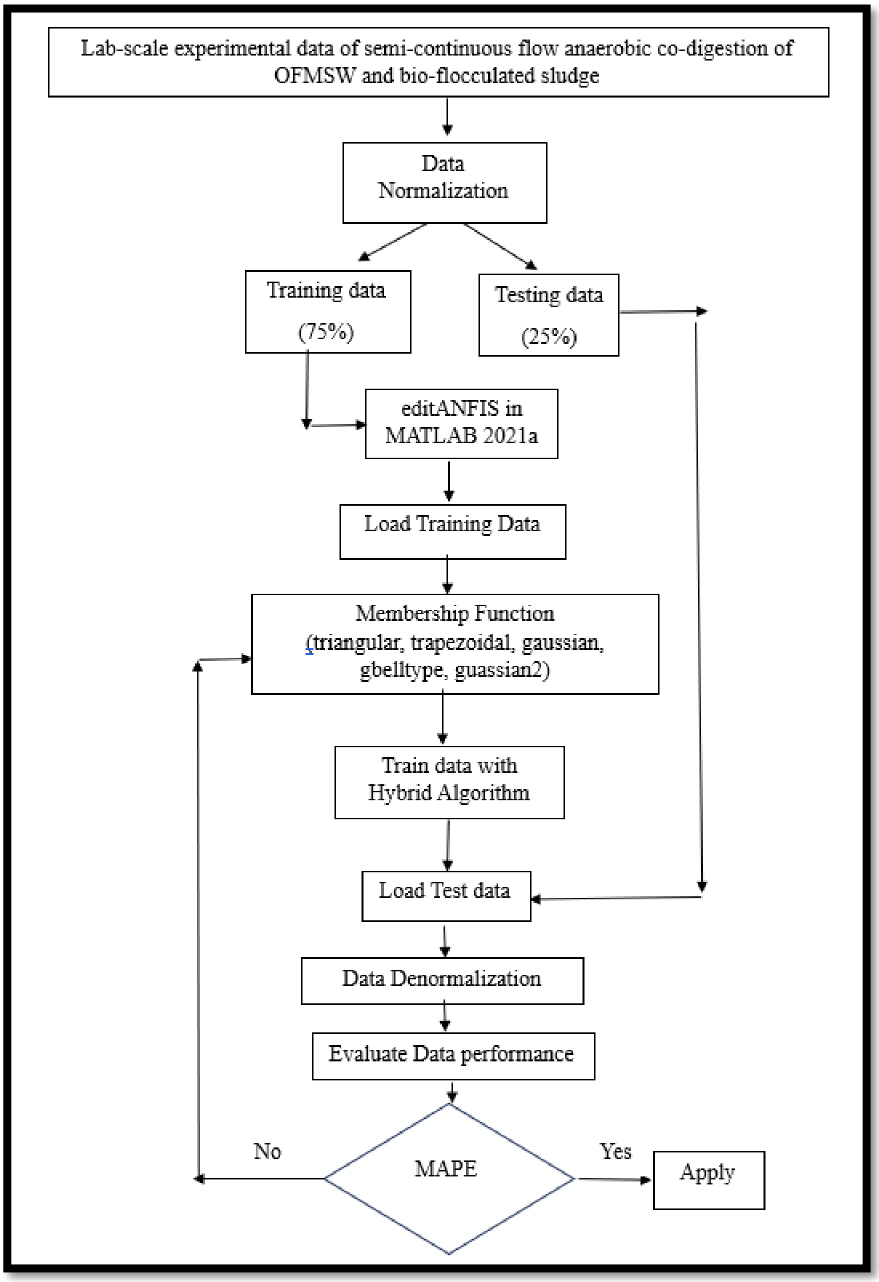

Figure 7: Process flow diagram for development of ANFIS model

3.9 Metagenomic Analysis

Metagenomic analysis is carried out to identify the dominant bacteria responsible for making the anaerobic co-digestion of OFMSW and bio-flocculated sludge efficient. A small-size acrylic reactor of capacity 1.2 L is fabricated. The substrate is fed with a mixing ratio of 75:25. The Analysis is carried out for genome sequencing using 16SrRNA methodology. The sample is collected in pre methanogenesis phase during the acclimatization phase of the reactor for the analysis. The fermentation or acidogenesis phase is also a very important phase of the anaerobic digestion process where presence of microbes leads to the success of the anaerobic co-digestion process.

3.9.1 Genomic DNA extraction and quality check

Approximately 10ml of sample is collected and stored at low temperature and a DNA sample is collected. The DNA quantity was measured with the help of NanoDrop ND-1000 spectrophotometer by determining A260/A280The quality of the quantified DNA is confirmed on 2% Agarose gel subjected to electrophoresis at 80 volts for 60mins. The quality of PCR is confirmed on the 2% agarose gel and V3-V4 primers are used for amplicon generation. Puregene NEX-GEN DNA Ladder is loaded along with the DNA and quality is checked. The library preparation is carried out by using Ultra II DNA lib preparation for Illumina Kit (NEBNext #E7645S/L). Library data found quality check pass hence sample is taken for further sequencing.

3.9.2 Methodology

Raw data quality assessment is performed and data is pre-processed using BBDuk v.38.86(qtrim=rl,trimq=18,tbo=f) (Mohsen et al.,2019). SILVA database (Release 138) is downloaded and sequences flanking the forward [CCTAYGGGRBGCASCAG] and reverse [GGACTACNNGGGTATCTAAT] primers are extracted. Stacked bar plots based on relative abundance using the Microeco R packages. Krona chart is generated using psadd (Annexure 6).

Table 7 depicts the section on material and methodology with a section on results and discussion available for the present study.

Table 7: Summary of experimental and analytical work

Section No.	Lab-Scale experimental work	Section under which results are discussed
3.6	**Batch Study**	**4.1**
	%TS &%VS pH Biogas yield COD concentration Mixing Ratio Kinetic parameters study	**(4.1.1 to4.1.8)** **4.2**
	Semi-continuous flow reactor anaerobic co-digestion	**(4.2.1 to 4.2.5)**
3.7	**Kinetic Modelling**	**4.3**
	First-order kinetic model	**(4.3.1 to 4.3.3)**
	Grau's second-order kinetic model	
	Modified Stover-Kincannon model	
	Modified Gompertz model	
	Logistic function model	
3.8	**Prediction modelling using ANN**	**4.4**
	Feed Forward Neural Network	**(4.4.1 to 4.4.3)**
	Relative Importance of Input Variable	
	ANN-PSO	
	ANFIS	
3.9	**Metagenomic Analysis**	**4.5**
	DNA extraction	**(4.5.1)**
	Methodology for genome sequencing	