

ABSTRACT

The rapid expansion of Indian cities, their economic development and population growth affect the generation of Municipal Solid Waste (MSW). It increasing concerns for the segregation of Municipal Solid Waste (MSW) lead towards more production of Organic Fraction of Municipal Solid Waste (OFMSW) in most cities of developing nations. OFMSW is the most favorable substrate in meeting goals of waste to energy in recent times using anaerobic digestion. The present study aims to improve anaerobic digestion process of OFMSW using co-substrate.

In developing nations like India, sewage is treated with Up-flow Anaerobic Sludge Blanket technology (UASB) where effluent needs to meet disposal standards. The treated effluent of UASB is conventionally treated with the Activated Sludge Process (ASP). In such plants bio-flocculated sludge generated from the Secondary Settling Tank (SST) needs special attention and its quantity is relatively less for the separate digestion requirement. Bio-flocculated sludge is rich in microbial biomass and is chosen for its potential to enhance methane production during the anaerobic co-digestion process. The use of bio-flocculated sludge aligns with the waste valorization principle where the bio-product of sewage treatment can be used as a potential co-substrate for the anaerobic co-digestion process.

Anaerobic co-digestion is the potential treatment for OFMSW as OFMSW needs liquification for a higher degradability rate and sludge being rich in microbial biomass enhances methane production during the anaerobic co-digestion process. The majority of Indian states have favorable meteorological conditions (20°C- 40°C for 9 to 10 months of a year). This integrated solution not only addresses the challenges associated with wastewater treatment but also leverages resource synergies to promote energy generation and environmentally conscious waste management practices.

The wet mass of OFMSW finalizes with 60-70% vegetable and fruit waste, 20-30% food waste, 0.5 to 1 % paper waste and 2-5% yard waste considered for lab-scale experimental work. OFMSW and bio-flocculated sludge mixed with different mixing ratios of 50:50, 75:25, 90:10, 100:0 & 0:100 (by % wet wt.). The mixing ratios of OFMSW: bio-flocculated sludge optimized using batch anaerobic co-digestion lab-scale experimental work. Anaerobic co-digestion exhibits a quick phase of acclimatization and raises methane production. With anaerobic co-digestion, the maximum specific methane gas yield is 369.28 ± 55.51 L/kgVS_{added} against 167.78 ± 16.45 L/kgVS_{added} in batch experimental mono-digestion of OFMSW. Lab-scale experimental data are used to validate the anaerobic co-digestion process of OFMSW and bio-

flocculated sludge using kinetic modelling. The kinetic models using the Modified Gompertz model and the Logistic Function model for methane production demonstrate the acceptance of anaerobic co-digestion batch experimental methane yield with R^2 0.99 for a mixing ratio of 75:25 with a high methane production rate and higher hydrolysis rate constant.

An optimized mixing ratio of 75:25 (% wet mass) of OFMSW and bio-flocculated sludge is used to perform lab scale experimental study using a semi-continuous flow anaerobic reactor. The reactor is operated with varied Organic Loading Rates (OLRs) with slight variations in composition and characteristics of OFMSW to reflect actual field conditions. The OLR is systematically varied within the range of 2,3,4,5,6 and subsequently 8 & 12 gm VS/L/d. The operation of the anaerobic mesophilic reactor consistently demonstrated fluctuation in biogas yield, pH, VFA/Alkalinity ratio and % VS removal even at the same OLR. This variation is attributed to change in the characteristics and composition of the substrate utilized for the anaerobic co-digestion process. %TS played an important role in the performance of the anaerobic reactor which is varied for the same OLR and different OLRs which affect the operation condition of the anaerobic reactor. Throughout the fluctuating %TS range and varying loading rate reactor performed well with a range of OLR 2 to 3 gm VS/L/d. The Optimum OLR for anaerobic reactor performance for OFMSW and bio-flocculated sludge is 3 gm VS/L/d due to a higher biogas yield of an average of 37.20 L/gm VS_{consumed} with lower VFA/Alkalinity ratio of 0.19 ± 0.06 , pH 6.9 ± 0.5 , %VS removal efficiency of 73.9 ± 1.9 during the operation of the reactor. A new hybrid kinetic model that combines the first-order kinetic model and logistic function is developed and applied to experimental data, predicting more accurate cumulative biogas yield with R^2 0.992 compared to other kinetic models. The Modified Stover-Kincannon model, Grau's Second-Order model and the First-Order kinetic model are applied for substrate removal efficiency prediction. Maximum removal rate constant (U_{max}) and saturation constant (K_B) achieved 31.74(gm/L*d) and 86.02 (gm/L*d) using the Modified Stover-Kincannon model. Grau's Second-Order kinetic constants a and b are 0.58 and 3.1 respectively. The First-Order kinetic model rate removal constant k_r is 0.12. The finding of the study demonstrates the value of kinetic models as a tool for improving the control of the anaerobic co-digestion process.

The development of the best prediction model depends upon the choice of appropriate modelling technique for the anaerobic co-digestion process. The operational, substrate quality and process control parameters such as OLR, Hydraulic Retention Time (HRT), pH, VFA/Alkalinity ratio and TS are input variables and methane yield and VS removal are output

variables for ANN modelling. The network architecture is optimized to achieve accurate predictions, resulting in a 5-19-2 architecture for methane yield and a 5-17-2 architecture for % VS_{removal}. The training is performed using the Bayesian Regularization (trainbr) algorithm, leading to high coefficients of determination (R^2) of 0.953 and 0.978 for methane yield and % VS_{removal}, respectively. The results demonstrate the effectiveness of ANN-based modelling in capturing complex relationships with the methane yield process, facilitating accurate prediction of crucial output parameters. Two sophisticated modelling approaches Artificial Neural Network-Particle Swarm Optimization (ANN-PSO) and Adaptive Neuro-Fuzzy Inference system (ANFIS) is used to predict methane yield in anaerobic co-digestion of OFMSW with bio-flocculated sludge. The hybrid approach captures intricate and non-linear relationships among input variables and methane yield using the ANN-PSO model achieves an R^2 of 0.80. ANFIS further elevates the modelling precision by leveraging linguistic variables and membership functions to optimize prediction with R^2 0.90.

Metagenomic analysis using 16SrRNA genome sequencing shows the abundance of Firmicute and Bacilli at the phylum level, Clostridia and Bacilli at the class level, Lachnospiraceae and Lactobacillaceae at the family level and Anaerostignum, Lactobacillus and Clostridium sensu stricto at genus level found. The capabilities of Lachnospiraceae to metabolize the polysaccharides, aromatics, and proteins that compose lignocellulose make them candidates to transform low-cost, sustainable, lignocellulosic feedstocks into value-added biochemicals. Lachnospiraceae and methanogens or acetogens have the potential for the direct conversion of lignocellulose to methane and other value-added biochemicals. The Anaerostignum genus found in abundance is of the same order level of Lachnospirales of the Lahnospiraceas family and from Firmicutes phylum.

This study contributes to advancing waste-to-energy practices as a solution for integrated solid-liquid waste management offering valuable insights for the potential use of bio-flocculated sludge from SST (post-UASB) as co-substrate for anaerobic co-digestion of OFMSW for the effective management of OFMSW. The lab-scale process employed in this study has the advantage of simplicity & economic affordability for replicating its use in large-scale plants in developing nations. The developed prediction model can be used to predict methane yield and % VS_{removal} efficiency with varied substrate characteristics for large-scale plant operation. The prediction model developed with ANN with curve fitting application provides more accurate modelling compared to traditional kinetic modelling for Volatile Solids removal using a modified Stover kin-cannon model. Further data integration will fortify the

accuracy and applicability of the proposed prediction models using ANN. The presence of the Lachnospiraceae family and Anaerostignum genus in abundance shows the potential of OFMSW and bio-flocculated sludge from SST (post-UASB) as a substrate to improve the anaerobic co-digestion process.

Keywords: Organic Fraction of Municipal Solid Waste, Bio-flocculated sludge, Anaerobic co-digestion, Kinetic modelling, Stover Kin-Cannon Model, Modified Gompertz Model, Artificial Neural Network

Chapter – I INTRODUCTION

1.1 Background

Population growth and the nation's economic situation both affect the generation rate of municipal solid waste. One of the factors influencing the rate of solid waste generation is education and awareness in society. The need for energy consumption rises as the population grows. Non-renewable energy sources are rapidly running out and they also cause more pollution in the nation, which harms the environment and living things. To meet future energy needs and protect the planet from the harmful effects of climate change, the usage of renewable energy must be increased. India's urban population has increased by 46.9 million (about 35.69% of the country's overall population). The rate of production of MSW is 150 t/d of which 27.08% is disposed of in landfills and 25.8% is unaccounted for contributing to the release of greenhouse gases (CPCB Annual Report, 2020-2021). Approximately 2.01 billion tons of MSW were produced in 2016 and that number will rise to 3.40 billion tons in 2050 (What a Waste 2.0). Urban local bodies (ULBs)/Municipal Corporations in India are currently focusing on improving the infrastructure and management practices to meet the future goals for sustainable practices in the field of MSW management. A significant portion of municipal solid waste (MSW) is comprised of biodegradable organic matter which is also known as the organic fraction of municipal solid waste (OFMSW). This includes cooked or uncooked food waste, vegetable and fruit peels, rotten vegetables and fruits, paper waste, and yard waste (Ahmed et al., 2022). If this OFMSW is disposed-off in a landfill without treatment, it will naturally decay and produce several greenhouse gases. Due to its biodegradability, waste generated in major Indian cities has the potential to be used in anaerobic digesters leading to the production of energy. The generation rate of biodegradable waste is significant in Indian cities ranging from 41% to 52% of the total MSW (Saini et al., 2012). It is possible to separate the Organic Fraction of Municipal Solid Waste (excluding plastic waste) and treat it using composting, vermicomposting, incineration or anaerobic digestion. For a growing nation like India, anaerobic digestion is one of the most preferred processes with little investment and potential revenue generation. The composition and quantity of solid waste generation vary from place to place and depend upon average income group, population, social behaviour, seasonal condition etc. (Khajuria et al., 2010), Minghua et al., 2009, Banerjee et al., 2019). The rate of production of Municipal Solid Waste in India is 160038.9 TPD of which 50% is treated, 18.4% is disposed-off in landfills and 31% is unaccounted for contributing to the release of greenhouse

gases. The accounting of biodegradable waste is significant in Indian cities ranging from 41% to 52% of the total MSW (Saini et al., 2012b; Sharma & Jain, 2019; Singhal et al., 2022).

Increasing population growth increases the sewage generation rate. There are 1093 sewage treatment plants in operational condition in India which can treat approximately 20235 MLD of sewage (CPCB annual report, 2021). Different treatment technologies are used to treat domestic sewage like ASP and Sequential Batch Reactor (SBR) are the most prevailing technology followed by UASB are used by most of the ULBs. In developing nations such as India, the utilization of UASB treatment technology for sewage treatment presents a strategic advantage owing to its low initial setup costs, economical operational maintenance and its potential for energy generation. However, the discharge of treated sewage using UASB necessitates secondary treatment, typically through the ASP to ensure compliance with inland surface water disposal standards specific to Indian conditions. According to the National Inventory of Sewage Treatment Plant CPCB, 2021 there are 76 UASB-based sewage treatment plants treating 3562 MLD of sewage in India. Post UASB when sewage treated with ASP also generates bio-flocculated sludge as a by-product that needs attention for its treatment and disposal.

1.2 Need of the study

For a growing nation like India, anaerobic digestion is one of the most preferred processes due to its cost-effectiveness, requiring low investment and having the potential for revenue generation. Anaerobic digestion of OFMSW has issues such as large particle size, high solids, slow biodegradation, lignin-rich waste and heterogeneous nature of the waste makes the process challenging (Hartmann & Ahring, 2005). OFMSW is considered as high TS substrate of approx. 35-50% leads to failure of the reactor performance due to mechanical problems, toxic substance accumulation, process inhibition due to higher acidification, low biogas yield, high C/N ratio, deficiency of macro and micronutrient, low degradation rate increases retention time which requires larger reactor volume (Cecchi et al., 2011). OFMSW must be treated with other co-substrates to enhance the waste-to-energy process. The process of AD allows the OFMSW to be microbiologically digested under anaerobic conditions to produce biogas which is rich in methane. The digestate can be used as an agricultural fertilizer (Chiu & Lo, 2016) (Vasumathi A.M.).

The sludge produced in the Secondary Settling Tank (post-UASB) is less in quantity and with poor porosity, cannot be dewatered easily, is low in solid content and therefore may be difficult

to dispose-off in conventional sludge drying operations. This sludge must go through some process to reduce volume, improve its characteristics, reduce health problems and hinderance to meet disposal standards and acceptance. The bio-flocculated sludge produced in the SST (post-UASB) has an abundance of microorganisms that can enhance the anaerobic digestion process. The prime use of bio-flocculated sludge from SST (post-UASB) for the anaerobic co-digestion process still needs to be focused based on various parameters and availability in Indian conditions to enhance the anaerobic digestion process of OFMSW. The development of an analytical prediction model can help to improve reactor performance and operational issues for on-field conditions.

The study of anaerobic co-digestion of OFMSW and bio-flocculated sludge from SST (post-UASB) is required to check the synergistic effect that can arise by using solid-liquid waste to enhance bio methanation process and nutrient recovery in the form of digestate which can be used for land application. This study underscores the potential of integrated waste management practices for sustainable and economical solutions (Figure 1).

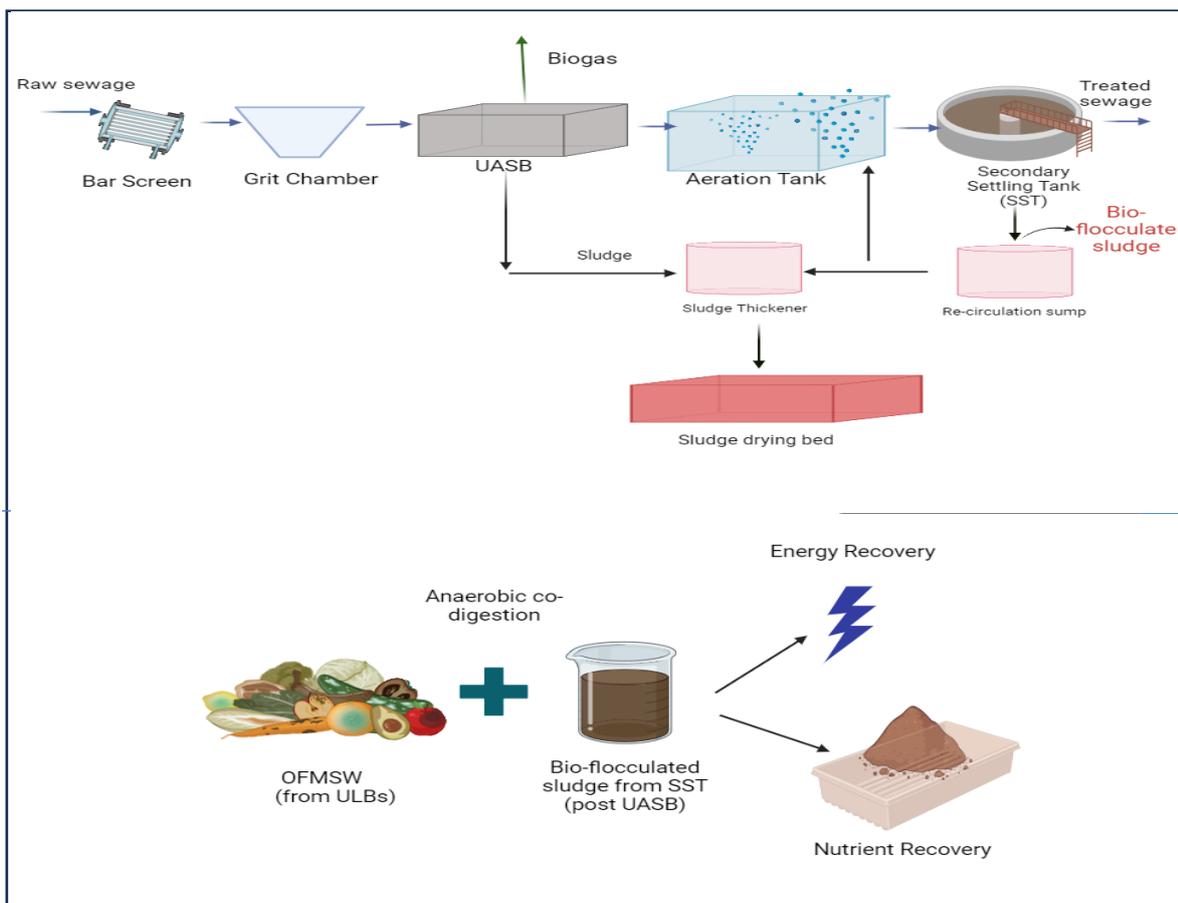


Figure 1: Substrate use for anaerobic co-digestion process

1.3 Objectives of Study

- To Check the feasibility of an anaerobic process for the co-digestion of OFMSW & bio flocculated sludge generated in ASP (post-UASB) of conventional sewage treatment in Indian conditions.
- To identify optimum conditions for various parameters to achieve satisfactory degradation and gas production for the co-digestion process.
- To scale up an analytical model for running a treatment scheme as suggested.

Chapter II - LITERATURE REVIEW

2.1 Findings from literature review

Various research has been carried out for the anaerobic co-digestion process. Major factors that help in the success of the anaerobic digestion process are substrate utilization, reactor design, operational condition etc.

Shahab & Anjum, 2022 study the factors affecting Municipal Solid Waste generation in Indian cities. India has 53 metropolitan cities that provide housing more than 45% of the urban population. Waste generation is directly proportional to population growth. The waste generation rate (kg/capita/day) is projected 0.56 for the lower-income group, 0.79 for the lower middle-income group, 0.99 upper middle-income group and 1.87 for the upper-income group by 2050. This clearly shows urbanization is the main factor for increasing the waste generation rate. This shows ULBs need to find the cost-effective and revenue generative solid-liquid waste management problem.

(Dai et al., 2019) study the effectiveness of particle size on biogas production. Rice straw from the field was taken to the laboratory and only the stem was used and nodes were removed. The particle size was reduced from 20mm to 1mm, 0.15mm and 0.075mm. Particle size reduction of rice straw improved methane yield from 107 ml/gm VS to 197 ml/gm VS. This study shows comminution pretreatment improved the basic morphology, dissolution ability and bio-liquefaction which helps in the shifts of bacteria community and decreased bacterial diversity.

Kumari et al., 2018 experimented anaerobic co-digestion process with a UASB reactor with four co-substrates added with a 1:2 mixing ratio with Kitchen Waste + Sewage Sludge (KW+SS), Yard Waste+ Sewage Sludge (YW+SS), Food Waste + Cow Manure (FW + CM) and Dairy Wastewater + Cow Manure (DWW+CM). The study shows anaerobic co-digestion is the most efficient and stable process for biogas yield with some challenges.

Smith & Almquist, 2014 observe that a study of anaerobic co-digestion of Food Waste (FW) with horse Manure using a Lab-scale two-phase mesophilic reactor will provide valuable insights. The second phase reactor was operated as the methanogenesis phase. 5ml of filtrate from the phase I reactor was transferred to the corresponding anaerobic reactor and the first feed of 2nd phase reactor was considered as the initial of the reactor performance. Phase 2 reactor generated more than 80% of the theoretical maximum methane yield. This study shows

that a two-phase anaerobic reactor helps to maintain acidogenic bacteria which improves the anaerobic digestion process.

(Ahmadi-Pirlou et al., 2017) Organic Fraction of Municipal Solid waste and WAS from dewatering pool anaerobically co-digested can result in low methane yield with High Solids (15%-20%) compared to low solids (5%-10%) content and with 5% TS maximum biomethane yield of 337 N mL/g VS achieved. This study shows substrate quality also impacts biomethane yield, especially total solid concentration.

Mougari et al., 2021 collected data from several published work-related mono-anaerobic digestion and anaerobic co-digestion of different organic waste. 42 samples were collected from different lab-scale experimental anaerobic digestion processes done under optimum environmental conditions. The prediction model was developed for cumulative biogas yield (CBY) and cumulative methane yield (CMY) using a multilayer perceptron algorithm (MLP) and modified Gompertz Model (MG). The study shows GA-MG required should be developed for each substrate mixture for prediction and that is a time-consuming process while the robustness of the ANN base prediction model for CBY and CMY over kinetic modelling can be an effective tool for scale-up of anaerobic digestion units and techno-economic studies.

Zhang et al., 2017 from a large-scale anaerobic digester and a sludge dewatering machine, inoculum and substrate were collected. Food waste was the co-substrate collected from canteen waste and homogenized by grinding. FW, WAS and inoculum were added in a ratio of 1:1:0.6 in the anaerobic reactor and co-pretreatment was provided with different time periods. Pyrosequencing analysis indicated a reduction in the abundance of filamentous bacteria of genus *Levilinea* in the co-digestion process which improve the anaerobic digestion process to achieve higher methane yield. *Methanobacterium*, *Methanosarcina* and *Methanosaeta* were observed in archaeal taxonomy at genus level with 20.8%, 46.5% and 32.2% respectively. This study shows metabolic pathways of microorganism helps to know the performance of the anaerobic digester.

Rego et al., 2019 study on performance of biodigester to enhance biogas production. The swine manure (SM) collected from the swine breeding unit and rice husk was collected, dried, cut into 0.5-1mm length. The mixture of swine sewage and rice husk was provided OLR between 1 to 1.5 gmVS/L/d where rice husk was used to provide more carbon source and added in a proportion of 2% wt. To predict the volume of biogas Artificial Neural Network (ANN) and Adaptive Neuro Fuzzy Inference System (ANFIS) were used. This study shows ANN with R2

0.77704 and ANFIS with R2 0.81209 having the capacity to predict biogas volume at its best configuration where ANFIS shows better performance than ANN.

Chapter – III MATERIAL & METHODOLOGY

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 high energy content (Aichinger et al., 2015; Bolzonella et al., 2006; Kumar & Samadder, 2017). MSW generated from cities comprises a maximum of organic fraction. OFMSW has high organic content and excellent biodegradability. Therefore, it can be treated using an anaerobic digestion process which is an eco-friendly treatment technology and useful for recovering energy from waste.

3.1 Characteristics and Composition of OFMSW and Bio flocculated Sludge from SST (post-UASB)

The composition of OFMSW is highly dependent on regional, seasonal, socio-economical, geographical conditions, availability, culture and usage of local products etc (Iacovidou et al., 2012; Tyagi et al., 2018). MSW comprises maximum bio-degradable waste of 42-53% which includes fruits & vegetable waste & food waste, (Katiyar et al., n.d.; Sharholly et al., 2007; Sharma & Jain, 2019; Thitame et al., 2010; Vinodbhai Mewada et al., 2020). Tier 1 to Tier 4 cities of India according to population size can generate an average of 41-52% of biodegradable waste (Saini et al., 2012b). In the present study, OFMSW takes into consideration wet waste (without plastic) (Figure 2) including vegetable and fruit wastes, vegetable and fruit peels, food trash, yard garbage, 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 for lab-scale 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 2: Organic Fraction of Municipal Solid Waste (OFMSW) for lab-scale experimental work

Physico-chemical characteristics of OFMSW are given in Table 2 which was utilized for lab-scale experimental work

Table 2: Physico-chemical Characteristics of OFMSW

Parameters	OFMSW
pH	5.8±0.4
%TS ^a	18.17±2.14
%VS ^b (of TS)	93.81±0.15
%Moisture content	81.82±0.86
COD ^c (mg/gm)	265.6±16.93
TKN ^d (mg/gm) (dry basis)	10.18±1.95
PhosphatePO ₄ ³⁻ (mg/gm) (dry basis)	1.16±0.28
%TOC ^e (dry basis)	50.59± 1.36

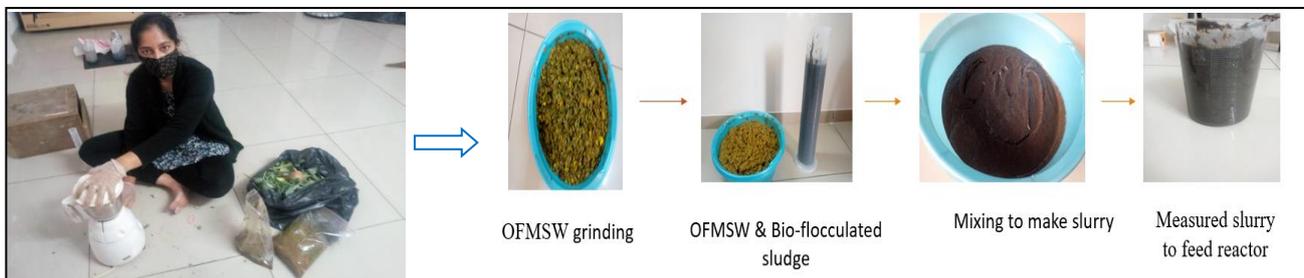
^a TS, Total Solids, ^b VS, Volatile Solids, ^c COD, Chemical Oxygen Demand, ^d TKN, Total Kjeldahl Nitrogen, ^e TOC, Total Organic Carbon

Table 3 provides Physico Chemical Characteristics of this bio-flocculated sludge generated from the Secondary Settling Tank (post-UASB) process. The bio-flocculated sludge generated from SST (post-UASB) rich in nutrients is the most prominent co-substrate for anaerobic co-digestion of OFMSW.

Table 3 : Physico-Chemical characteristics of bio-flocculated sludge from SST

Parameters	Bio-flocculated sludge from SST
pH	7.9±0.2
%TS ^a	5.24 ± 1.18
%VS ^b (of TS)	53.97±0.92
%Moisture content	94.76±0.6
COD ^c (mg/gm)	52.22±4.03
TKN ^d (mg/gm) (dry basis)	4.75±0.36
PhosphatePO ₄ ⁻³ (mg/gm) (dry basis)	4.89±1.08
%TOC ^e (dry basis)	28.52±1.52

Pre-treatment of OFMSW is required to improve the anaerobic co-digestion process. Pre-treatment can be given to the substrate with various techniques like mechanical, chemical or biological, biochemical, thermal, alkaline and hybrid methods (Dai et al., 2019, Rabii et al., 2021). The size reduction of particles enhances the substrate utilization rate by the anaerobic microbes (Kim et al., 2000). Apart from process efficiency, the economic (reduced cost) and environmental (smaller carbon footprints) are the factors that determine the success of the pretreatment method (Tyagi et al., 2018). The simplest and economically viable pretreatment method applied for the present study is cutting and grinding. The OFMSW is grinded to a size of 2 mm ~ 5 mm. Grinding enabled the increase in surface area available for microbes. Figure 3 describes the substrate preparation for anaerobic co-digestion for lab-scale experimental work.

**Figure 3: Pretreatment of OFMSW and feed preparation**

Substrate characteristics of OFMSW and bio-flocculated sludge with different mixing ratios are shown in Table 4. It is observed that when OFMSW is mixed with bio-flocculated sludge

at different mixing ratios can help to improve substrate characteristics which can improve operational conditions for the anaerobic co-digestion process.

Table 4: Physico-chemical characteristics of OFMSW & Bio-flocculated sludge with different mixing ratio

Substrates →	50:50	75:25	90:10	0:100	100:0
Parameters					
pH ↓	6.37±0.22	6.16±0.12	6±0.18	8.08±0.1	5.84±0.21
%TS ^a	6.96±0.27	15.26±0.44	18.5±0.38	5.05±0.20	19.04±0.26
%VS ^b (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 ^c (mg/gm)	168.96±2.28	191.8±0.14	185.6±3.7	52.24±3.07	256.38±4.52
TKN ^d (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 ³⁻ (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 ^e (dry basis)	45.21±1.12	49.86±1.82	47.10±2.44	30.77±0.12	51.31±0.028

3.2 Experimental set -up for anaerobic co-digestion process

A batch anaerobic reactor (Figure 4) made up of an acrylic sheet with a volume of 10L is used. The substrate is fed on a wet mass basis. During the study, each batch contained a total of 7 kg of feed. Complete mixing of the substrate is achieved using a stainless-steel mixer with paddles operated at low speed (intermittent only) with a 12V DC motor. A water jacketing system with heating rods is supplied around the batch reactor which can effectively maintain the temperature inside the reactor (30-35°C). Utilizing the water displacement method, biogas produced during batch study is quantified. The water displacement unit absorbs the CO₂ gas produced during the anaerobic process by passing biogas through NaOH solution. 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.

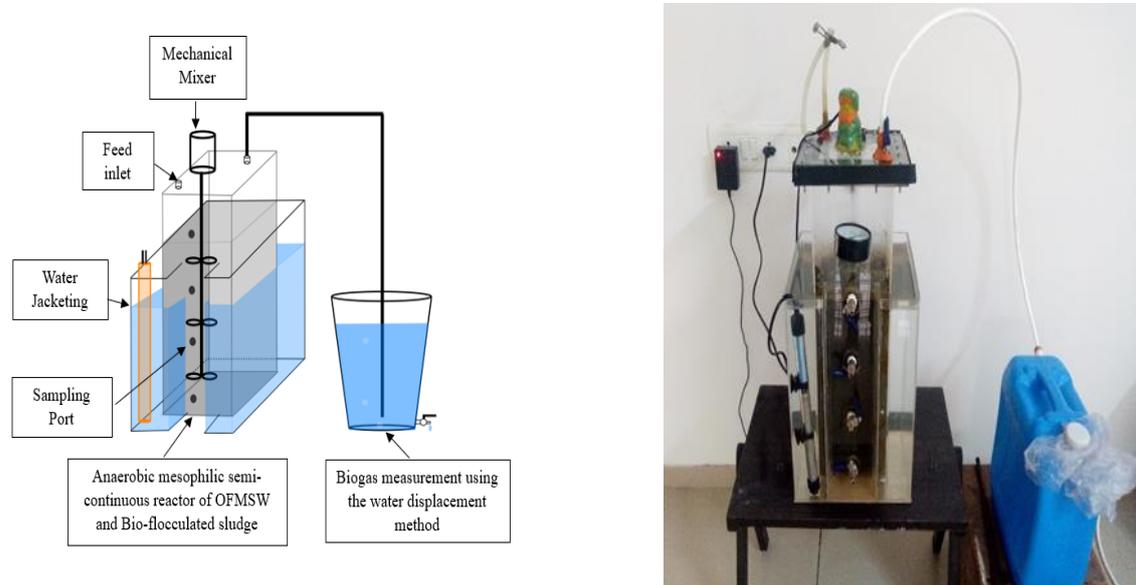


Figure 4: Schematic diagram of the lab-scale experimental setup

3.3 Analytical Method

For the measurement of pH, samples are centrifuged (Clinical Centrifuge, model 858/8, REMI electricals, @3500 rpm-10 minutes). Total Solids and Volatile Solids are measured regularly (2540-G). For the measurement of COD, a closed-reflux titration method is used (5220-C). Sample preparation for COD is carried out using 1 gram of a wet sample acidified with 2 ml of concentrated H₂SO₄ and the sample volume is made up of 10 ml of distilled water. To measure the volume of methane gas, the water displacement method is used with NaOH solution to absorb CO₂ gas. Total Kjeldahl nitrogen and Total Phosphate is measured with the Macro Kjeldahl method and Stannous Chloride method (4500 N_{org}-B, 4500P-D) respectively. The volatile Fatty Acids to Alkalinity ratio is measured with Kapp's method. 20 to 30ml of centrifuge sample is taken for analysis of initial pH. Then the sample was titrated slowly with 0.1N H₂SO₄ until reached pH 5,4.3, and 4. Equation (1) and (2) show measurement of VFA and Alkalinity.

$$\text{Alkalinity} = A * N * 1000 / \text{Sample Volume} \quad \text{Eq. (1)}$$

$$\text{VFA} = 131340 * N * B / \text{Sample volume} - 3.08 * \text{Alk} - 10.9 \quad \text{Eq. (2)}$$

Where,

Alk= Alkalinity (mmol/L), A= 0.1N H₂SO₄ 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), Alkalinity (mmol/L) *100 will convert into mg/L

3.4 Analytical Modelling Techniques

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

Kinetic studies can be used to forecast digester performance, digester design, and substrate biodegradability. The first-order kinetic model (Equation 3), Modified Gompertz model (Equation 4) and Logistic Function model (Equation 5) are used to model experimental methane production for all co-digestion mixing ratios. With IBM SPSS 2021, the model's kinetic constants for P_m, R_m, and λ are calculated using the least squares fitting method (non-linear regression methodology).

$$\text{First-order kinetic model: } M = P_m \times [1 - \exp(-kt)] \quad \text{Eq. (3)}$$

$$\text{Modified Gompertz Model: } M = P_m \times \exp -\left\{\exp \frac{[R_m \times e^{(\lambda-t)+1}]}{P_m}\right\} \quad \text{Eq. (4)}$$

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

Where,

M is the methane yield (L/kg VS_{added}) concerning time t (days), P_m is the maximum methane 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 methane production rate (L/kg VS_{added}*d), λ is the lag phase time (days), e is Euler's function = 2.7183.

3.4.2 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 the substrate removal rate.

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

$$S_e = S_i - \frac{U_{max} * S_i}{K_B + (Q * \frac{S_i}{V})} \quad \text{Eq (6)}$$

The Substrate removal rate is defined as follows:

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 value constant (gm/L*d); Q = flow rate (L/d); V = Volume of the reactor (L)

U_{max} and K_B can be calculated with the intercept and slope of the line respectively.

Grau's Second-Order equation (7) obtained is given as below

$$\frac{HRT}{E} = a + b * HRT \quad \text{Eq (7)}$$

Where, Substrate removal efficiency (E) = $(S_i - S_e) / S_i$; the kinetic constants a and b are the intercept and slope determined from the plot of HRT vs $HRT / COD_{removal}$.

$$\frac{S_i - S_e}{HRT} = kr * S_e \quad \text{Eq. (8)}$$

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); HRT is the hydraulic retention time (d)

The value of kr can be calculated by plotting S_e versus $[(S_i - S_e) / HRT]$.

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

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

The human brain is a complicated structure with a densely linked network of basic processing units, or neurons. The simplified representation of the organic nervous system is called an artificial neural network or neural network. An artificial neural network (ANN) is a computer learning system that uses mathematical relationships between input-output variables to discover the link between a set of defined input data and output data with a wide range of

operational conditions (A. Ramchandran,2019). An input layer, a hidden layer, and an output layer are the three layers are there in ANN. Input variable (%TS, OLR gmVS/L/d, pH, HRT, VFA/Alkalinity ratio) and output variable (% VS_{removal} and Methane yield (L/kgVS_{removed})) are included from the experimental data to develop prediction model.

3.4.3.1 Development of Neural Network prediction model using fitting application

ANN study is carried out with MATLAB 2021a to implement the Feed Forward training algorithm with the curve fitting application (fitnet). Levenberg-Marquardt, Bayesian Regulation, and Scale Conjugated Gradient are the three training algorithms employed in this work along with the tan sigmoid transfer functions.

To make the selected data acceptable for the activation function in the neural network, all of the data are scaled to the range [0-1] using the minimum and maximum values of each variable using Equation (9).

$$X_n = \frac{X - X_{\min}}{X_{\max} - X_{\min}} \quad \text{Eq. (9)}$$

X = experimental data

x_n = normalized 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 (2)

$$X_n * [(X_{\max} - X_{\min})] + X_{\min} = X \quad \text{Eq.(10)}$$

3.4.4 Relative Importance of input variable (RI)

To determine the association between input and output variables, ANN models are typically utilized. Connection weight method, Garson's algorithm, and other techniques employ 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.

3.4.5 Development of prediction model using ANN-PSO

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 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 neighbors' 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 optimization tool places a focus 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. 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.

3.4.5 Development of prediction model using ANFIS

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, 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 by the ANFIS toolbox on MATLAB. 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. ANFIS model evaluated with testing data set with lowest MAPE.

Chapter – IV RESULT AND DISCUSSION

The present chapter aims to describe the feasibility of anaerobic co-digestion of OFMSW and bio-flocculated sludge from SST (post-UASB) with batch experiment. The robustness and reliability of the performance of the anaerobic co-digestion process is checked by operating at a semi-continuous flow with varied OLRs. The performance results are validated with different kinetic models. The lab-scale experimental data are used to develop a prediction model using an Artificial Neural Network (ANN).

4.1 Anaerobic co-digestion of OFMSW & bio-flocculated sludge (post-UASB) with batch experimental study

To reach future targets for sustainable practices in the field of MSW management, Indian municipal corporations with urban local bodies (ULBs) are currently concentrating on enhancing the infrastructure and management practices. The OFMSW, also known as biodegradable organic matter, makes up a sizeable component of MSW. Food waste whether cooked or raw, fruit and vegetable peels, spoiled fruits and vegetables, paper garbage and garden waste are all included in this OFMSW (Ahmed et al., 2022). This OFMSW will decompose naturally if it is dumped in a landfill without treatment, it releases several greenhouse gases. Waste produced in large Indian cities has the potential to be utilized in anaerobic digesters to produce energy because of its biodegradability. Anaerobic digestion can be done in several ways, such as mono digestion, co-digestion with various substrates, dry/semi-dry batch systems, wet continuous systems, and dry anaerobic continuous systems etc (Charles et al., 2009). Two or more substrates are mixed in anaerobic co-digestion to take advantage of their complementary qualities and enhance the process outcome result (Prabhu & Mutnuri, 2016).

This study focuses on anaerobic co-digestion of OFMSW and bio-flocculated sludge from SST in batch reactors. The study takes into account several ratios (% wet weight) of OFMSW and bio-flocculated sludge (50:50, 75:25, 90:100, 0:100, and 100:0) to check the performance of the reactor. In the current sewage treatment plant, the sludge is recovered from UASB after sewage treatment is utilized (100ml) as an inoculant to initiate rapid startup.

4.1.1 Effect on %Total Solids

The Total Solid content is essential to get the most biogas possible from the substrate. A Total Solid concentration of < 12% yields the maximum amount of methane produced by the substrate (Borowski, 2015). Higher Total Solid concentration in substrate takes longer degradation time and due to the accumulation of VFA, it also decreases biogas yield(Liao et al., 2014). Different research has different outcomes regarding optimum %TS concentration and the success of the anaerobic digestion process also depends upon other parameters like substrate characteristics, environmental conditions, operational conditions. It is difficult to optimize the %TS of substrate but this factor is very important for the operation of the anaerobic digestion process so it must be considered during the anaerobic digestion process. According to available research and substrate composition TS concentration of OFMSW is taken into consideration for the present study.

In the present study, the TS content of OFMSW in anaerobic digestion is greater than 15%. The concentration of %TS is decreased when bio-flocculated sludge from SST is combined with OFMSW at different percentages of wet weight. When OFMSW is combined with 50% (of wet weight) bio-flocculated sludge from SST, the total solids content is less than 10%. Methanogens require less time to acclimatize to bio-flocculated sludge from SST, which also boosts particular biogas generation. When co-digested with varying mixing ratios, OFMSW and Bio-flocculated sludge from the SST process efficiently create methane at a rate roughly equivalent to that of the substrate with low TS content. One possible explanation for the lack of a discernible drop in T S concentration during the course of the experiment, as seen in Figure 5, could be the addition of NaHCO_3 alkali to raise the pH during the process.

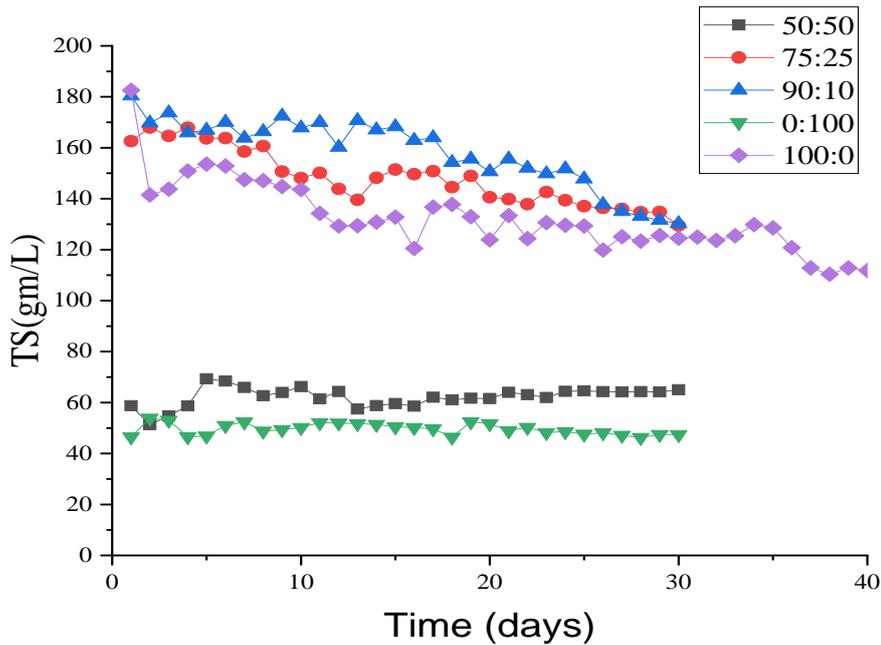


Figure 5: Effect on Total Solids(gm/L) during anaerobic co-digestion process

4.1.2 Effect of pH

Substrate breakdown occurs instantaneously in an anaerobic environment, causing a drop in pH and a concentration of volatile fatty acids. Ideally, VFA should be converted to CO_2 and CH_4 by an active mass of microorganisms; however, the development of methanogens is suppressed in the reactor due to the high concentration of organic substrate and buildup of VFA. Consequently, alkali (NaHCO_3) must be added on regular and constant basis to raise the pH in the range of 6.4 to 7.8.

It has been noted during the investigation that co-digesting OFMSW with bio-flocculated sludge from SST helps to shorten the time it takes for the methanogenesis bacteria to activate. The co-digestion mixing ratio of 50:50 (OFMSW: Bio-flocculated sludge) requires less time to stabilize the pH of the anaerobic reactor than does the anaerobic mono-digestion of OFMSW. The bio-flocculated sludge from SST (post-UASB) is the type of bio-flocculated sludge that may control pH in a few days without the need for alkali addition (Figure 6). Compared to the previous two scenarios, the anaerobic digestion of OFMSW alone resulted in the generation of higher acids and an increased need for alkali to stabilize reactor operations for methanogenesis.

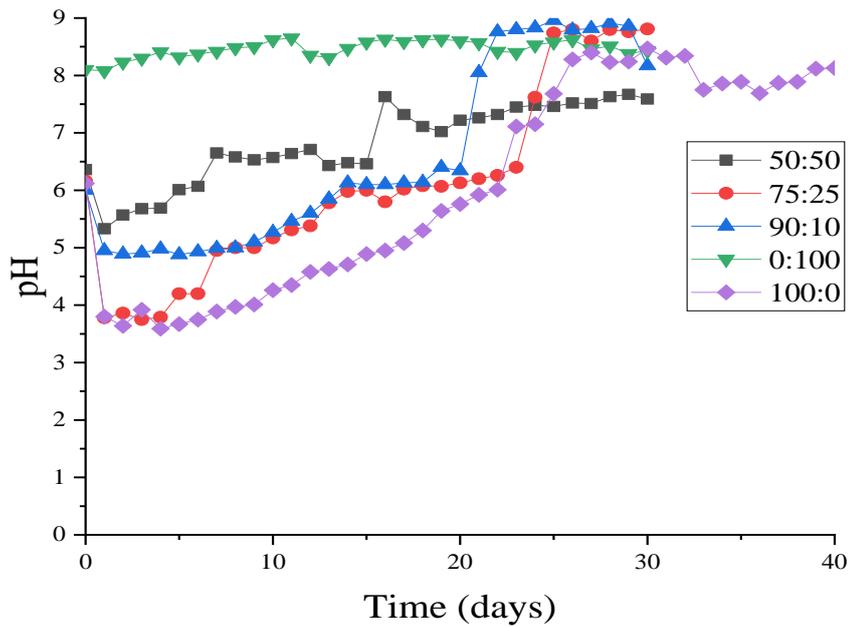


Figure 6: Effect on pH during anaerobic co-digestion process

4.1.3 Degree of degradation using Volatile Solids (%)

How well the anaerobic process operates is demonstrated by the degree of degradation. To calculate the substrate's efficiency based on volatile solids (of TS), the steady state condition is taken into consideration, meaning that only volatile solids from input and outflow are taken into account. Volatile Solids concentration evaluates the performance of the anaerobic digestion process, the potential of biogas yield and biomass degradation. In comparison to the co-digestion mixing ratio (50:50), the maximum VS elimination effectiveness is attained with co-digestion mixing (90:10) and (75:25) of 77.87% and 85.97%, respectively. The volumetric biogas yield is associated with a decrease in volatile solids concentration. Anaerobic co-digestion with high TS concentration and mono digestion has a similar reduction in VS (Figure 7). But when compared to mono-digestion of OFMSW about volatile solids reduction, specific biogas yield is higher with a mixing ratio of 50:50,75:25,90:10. This could be because there is a higher production of volatile fatty acids with more organic fraction, which inhibits the production of biogas. % VS_{reduction} is calculated with equation 11 (Konard Koch, 2015) where considering the inorganic solids are not degraded during the anaerobic digestion process.

$$\%VS_{removal} = 1 - \frac{VS_{out} * (1 - VS_{in})}{VS_{in} * (1 - VS_{out})} * 100 \quad \text{Eq. 11}$$

VS_{in} = Input Volatile solids of the substrate (gm/gm)

VS_{out} = Output Volatile solids of the substrate after anaerobic digestion (gm/gm)

Primary Sludge and fruit and vegetable waste anaerobically co-digestion under batch mesophilic condition, Volatile Solids removal efficiency was measured 73% and 70% for a mixing ratio of 70:30 and 50:50 respectively with maximum methane yield with a mixing ratio of 50:50 (Elsayed et al., 2019). Dry anaerobic co-digestion of Citrus waste, chicken feathers and wheat straw at a mixing ratio of 1:1:6 performs best with 238 ml/gmVS methane yield (Regina J. Patinvoh et al.,2018).

4.1.4 COD removal efficiency

The study's initial COD values for the Substrate bio-flocculated sludge from SST and OFMSW are observed to be 52.22 ± 4.03 mg/gm and 265.5 ± 16.93 mg/gm, respectively. This shows that the bio-flocculated sludge from OFMSW and SST is biodegradable on its own and may produce biogas; when co-digested, this can increase the output of biogas. The co-digestion of OFMSW and bio-flocculated sludge from SST results in a COD removal efficiency enhancement of $20.37 \pm 5.41\%$. Anaerobic co-digestion ratios of 50:50 and 75:25 show higher COD removal efficiency as compared to the mono-digestion of OFMSW. COD removal efficiency calculated with equation 12

$$\%COD_{removal} = \frac{COD_{in} - COD_{out}}{COD_{in}} * 100 \quad \text{Eq. 12}$$

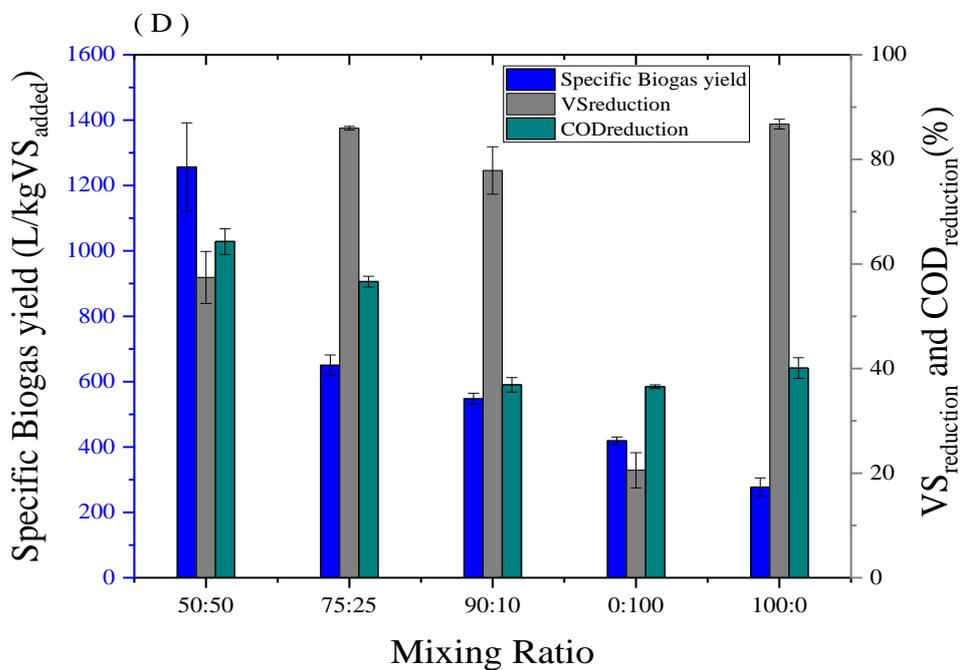


Figure 7: % VS reduction, %COD reduction, Specific Biogas yield (L/kgVS_{added}) for batch anaerobic co-digestion at different mixing ratios

Figure 7 depicts that a mixing ratio of 50:50 shows a high specific biogas yield which is quite higher than mono digestion. At the same time mixing ratio of 75:25 shows a higher % VS removal and high COD removal efficiency with comparatively high specific biogas yield.

4.1.5 Anaerobic reactor performance with ambient temperature condition

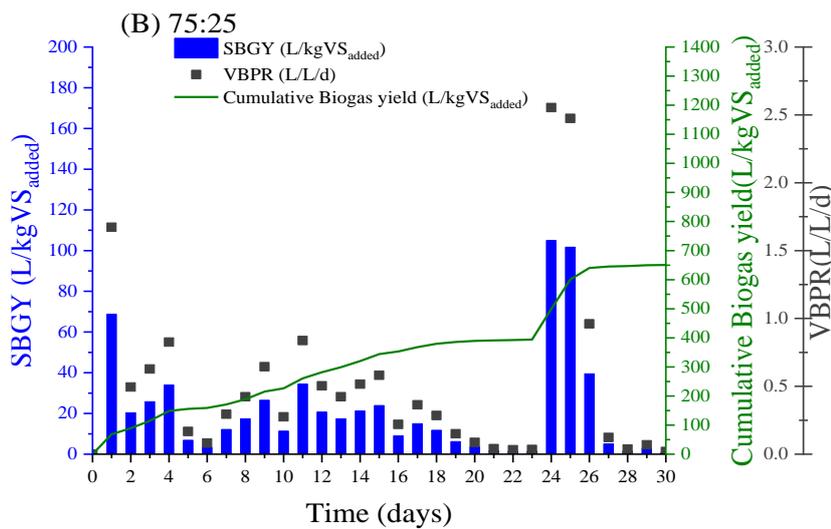
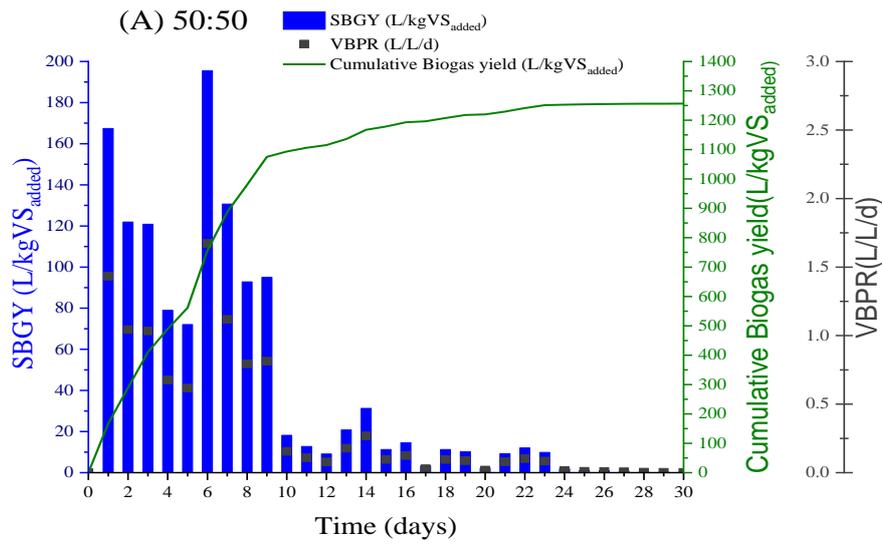
The temperature controllers in reactors are considered expensive and not preferred in developing countries like India. Hence, any study on anaerobic digestion must take into account these aspects to be of relevance in field application. The anaerobic reactor must be able to function in the ambient temperature conditions. India being a tropical country the operating temperature can be the ambient temperature for most of the months in a year. This mesophilic temperature range of 25 to 35 °C (Jain et al.,2015) assures that an uninterrupted anaerobic co-digestion process can be carried out for the supply of biogas throughout the year. The annual day temperature at the study area was observed 29 °C to 40 °C and during the night 17 °C to 29 °C (sometimes 12°C to 13 °C during winter nights). Maintaining this mesophilic condition for a few months of a year requires a temperature controller. Here in this study, the temperature of an anaerobic reactor was controlled with a water jacketing system and heating rod between 30 °C to 35 °C

4.1.6 Effect of co-digestion mixing ratio on biogas yield

More biogas is produced as the amount of bio-flocculated sludge from SST mixing rises. The yield of specific biogas production for the blending ratios of 50:50, 75:25, 90:10, 0:100, and 100:0 is 1257.05±135.28, 650.99±31.36, 548.38±16.98, 419.44±11.59, and 277.41±27.98 (L/kg VS_{added}), respectively. Figure 8 displays the cumulative biogas production (L/kgVS_{added}) together with the Specific Bio Gas yield (SBGY) and Volumetric Biogas Production Rate (VBPR) of the anaerobic digestion of OFMSW and bio-flocculated sludge from SST. Specific Biogas yield is determined by Biogas generated (L) per Volatile Solids supplied (gm/L) and volumetric biogas production rate (L/L/d) is the volume of biogas production (L/d) per volume of substrate in the reactor (L) during the anaerobic digestion batch experiment.

Anaerobic digestion produces two separate peaks for mixing ratios of 50:50, 75:25, 90:10, and 100:0, respectively (Dong et al., 2010). The first peak in the current study appears during the first five days of anaerobic digestion. It is followed by a second peak that appears on days seven, twenty-three, twenty-one, and twenty-four and steadily stabilizes until the end of the experiment. VFA is produced at start-up settings by the easily biodegradable substrate's hydrolysis and acidogenesis. The buildup of VFA, which is immediately detectable with a pH

decrease throughout the anaerobic process, is correlated with the first tiny peak. Previous research indicates that acetoclastic methanogen development is inhibited by the initial partial pressure of H_2 . The influence of partial pressure of H_2 may lessen later in the anaerobic process, allowing for the activation of more acetoclastic methanogens, which provide a second peak during the generation of biogas.



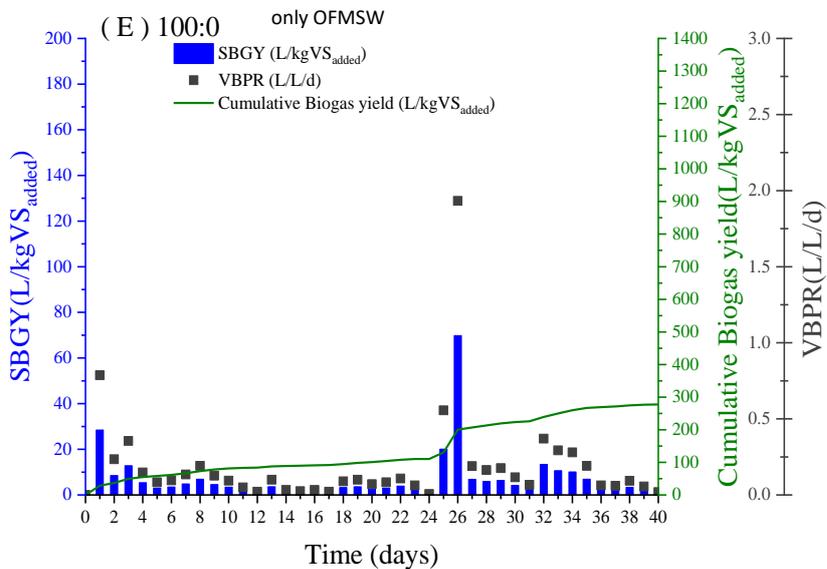
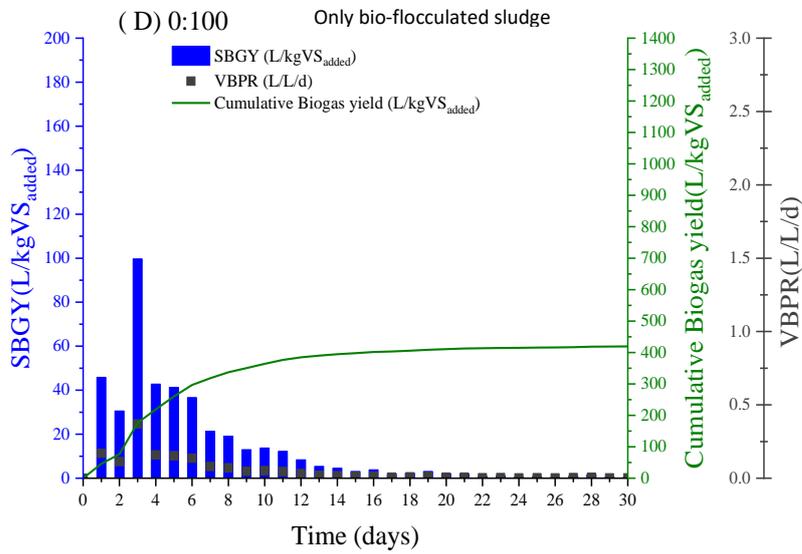
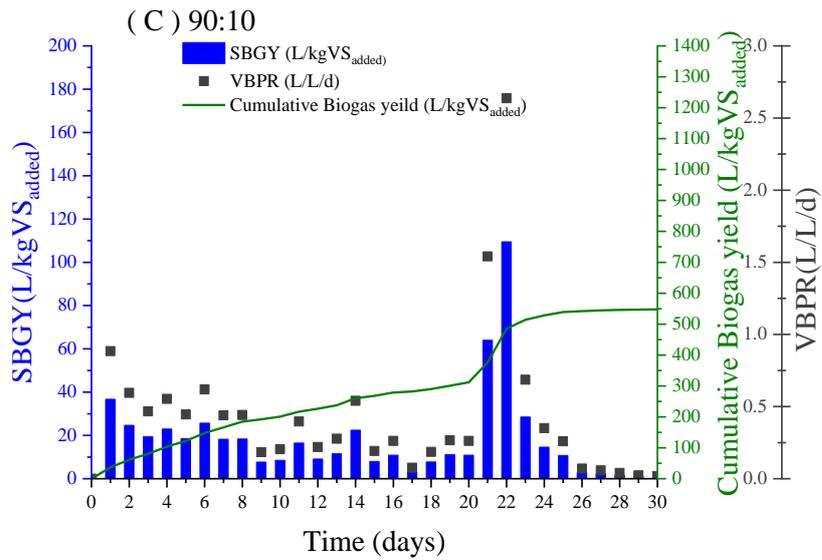


Figure 8: VBPR (Volumetric Biogas Production Rate) (L/L/d); SBGY (Specific Bio-Gas Yield) (L/kg VS_{added}); Cumulative Biogas Yield (L/kgVS_{added}) for different mixing ratios (A) 50:50 (B) 75:25 (C) 90:10 (D) 0:100 (Only Sludge) (E)100:0 (only OFMSW) of anaerobic co-digestion

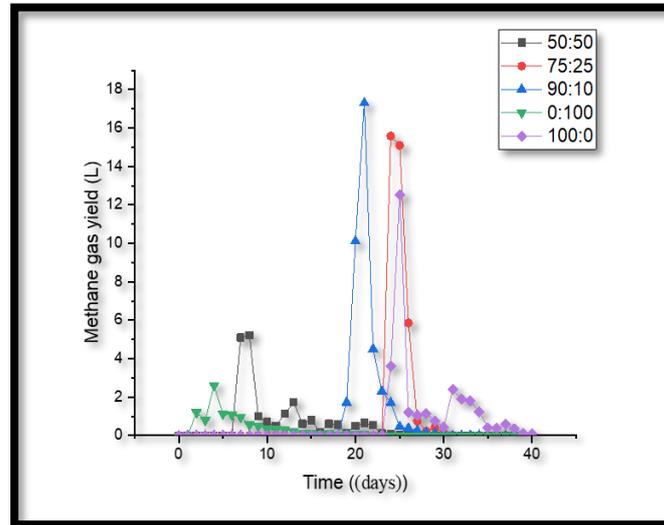
When biogas is ignited, the very pure methane gas produced at the second peak of the gas production process flares up blue (Figure 9). When bio-flocculated sludge from SST is anaerobically mono-digested, methane gas is generated over an extended period. The volumetric biogas production rate follows the trend of biogas generation. This demonstrates how the anaerobic co-digestion of OFMSW with bio-flocculated from SST with varied mixing causes a dynamic change in condition.



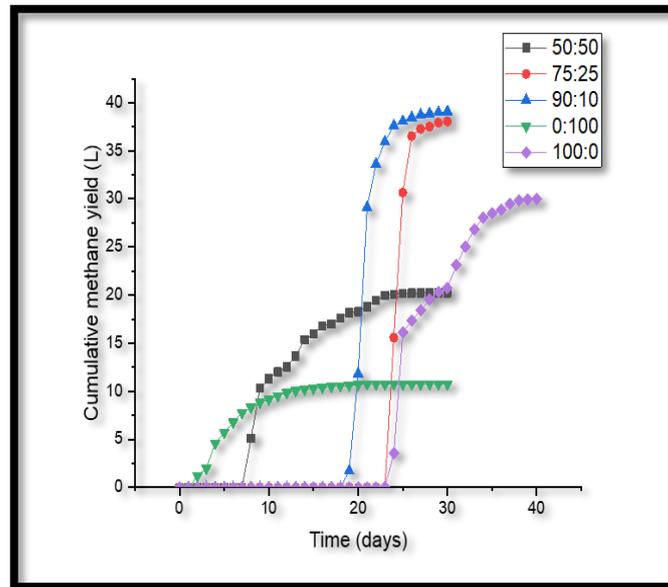
Figure 9: Presence of methane gas during anaerobic co-digestion

Methane gas is not likely to be present during the first start-up state. During the anaerobic digestion of batch experiments, the second peak of biogas production is considered the methanogenesis phase under mesophilic conditions. Therefore, the methane gas produced during this methanogenesis phase is taken into consideration in the specific methane gas yield (L/kgVS_{added}) and volumetric methane gas production rate (L/L/d). For the various mixing ratios of 50:50, 75:25, 90:10, and 100:0, the final methane yield is computed as 369.29 ± 55.51 , 256.44 ± 12.98 , 246.83 ± 46.23 , and 167.78 ± 16.45 (L/kgVS_{added}), respectively. Observations show that for bio-flocculated sludge from SST, the final methane output and specific biogas production are 419.25 ± 11.59 and 373.41 ± 36.32 (L/kgVS_{added}), respectively. OFMSW co-digestion yields more methane production than mono-digestion in this batch study on anaerobic digestion. Using an OFMSW and bio-flocculated sludge from SST mixing ratio of 50:50, 75:25, 90:10, 0:100, 100:0, higher volumetric biogas production and volumetric methane gas

production rates of 0.358 ± 0.038 , 0.527 ± 0.02 , 0.439 ± 0.013 , 0.0518 ± 0.006 , 0.192 ± 0.019 L/L/d and 0.137 ± 0.02 , 0.891 ± 0.04 , 0.540 ± 0.10 , 0.0477 ± 0.004 , 0.288 ± 0.028 L/L/d, respectively, are achieved. Methane gas generation for different mixing ratios is demonstrated in Figure 10.



(A)



(B)

Figure 10: (A) Methane yield (L/d) (B) Cumulative methane yield (L) for batch anaerobic co-digestion with different mixing ratio

These outcomes compare well to or surpass those of previous studies that used OFMSW co-digested with other co-substrates. The specific methane Yield of bio-flocculated sludge from SST (post-UASB) is $373.41 \text{ L/kg VS}_{\text{added}}$, which is comparable to the 249 mL/gm VS (Cabbai

et al., 2013). 369 mL/gm VS methane yields of sewage sludge. When nightsoil and food waste anaerobic in batch study co-digested with a mixing ratio of 70:30 can generate 0.38gmCH₄ COD /gm VSS per day (Khanto & Banjerdki, 2016), Brown water combined with food waste in the same proportion (1:1) and inoculum sludge from WWTP at biogas yield was 417 L/kgVS (Lim, 2011). OFMSW mixed with activated sludge and rice straw with 1:1.5:1.5 in the batch study under the temperature of 37 C achieved 339.9 L/kgVS biogas yield. Food waste co-digested with septic tank sludge (75:25) and cow dung as inoculum results in 471ml/gm VS (Kesharwani & Bajpai, 2020). In batch anaerobic co-digestion under mesophilic conditions food waste and sludge (before centrifuge) when co-digested and as inoculum effluent from AD treating Food waste is utilized, 823ml/gmVS biogas generated (Prabhu & Mutnuri, 2016).

4.1.7 Microscopic and SEM imaging of methanogens

Methane is produced during anaerobic digestion by methanogens. Several methanogen species were investigated during the anaerobic procedure in previous studies where acetoclastic methanogens, species of both Methanosarcina and Methanosaeta are typically present while CO₂-reducing methanogens include Methanobacterium, Methanothermobacter, Methanobrevibacter, Methanogenium, Methanocorpusculum, and Methanospirillum species were present. Microscopic image (Olympus-BX53) & SEM (Scanning Electron Microscopy) photographs of the methanogens are obtained in this investigation to verify their presence. During the methanogenesis phase, sampling is carried out for microbial analysis. To get a distinct image of methanogens and their form image is taken with a JEOL JSM-6380LV Scanning Electron Microscope. Figure 12 depicts the rod shape of methanogens with flat ends. According to its forms, it indicates the likelihood of Methanosaeta existence (Kim & Whitman, 2014) also known as Methanothrix is found to be the most abundant methanogen which had increased largely along with the process of methanogenesis. This order is considered to be the majority contributor to methane production through the acetoclastic pathway producing methane by converting not only acetic acid but also capable of utilizing all methanogenic pathways to produce biogas (Arelli et al., 2021)

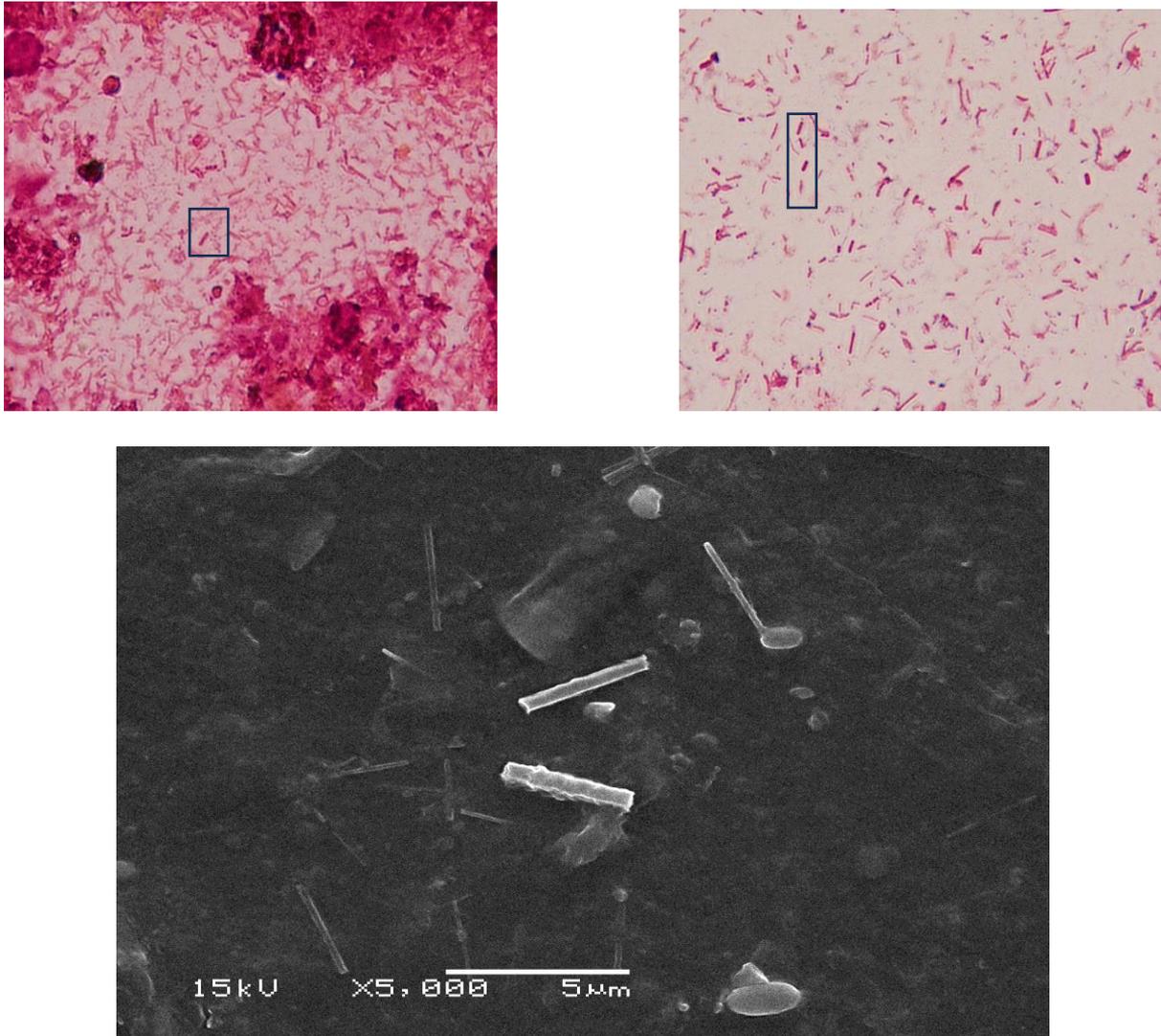


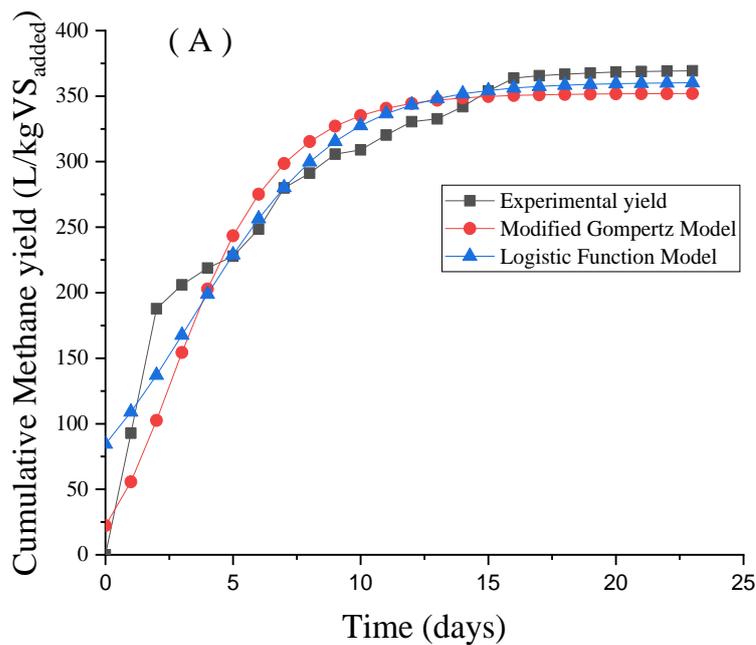
Figure 11: Microscopic analysis (A) Methanogens in whole Sample (B) Methanogens in Supernatant (C) SEM (Scanning Electron Microscope) image of Methanogens during the methanogenesis phase

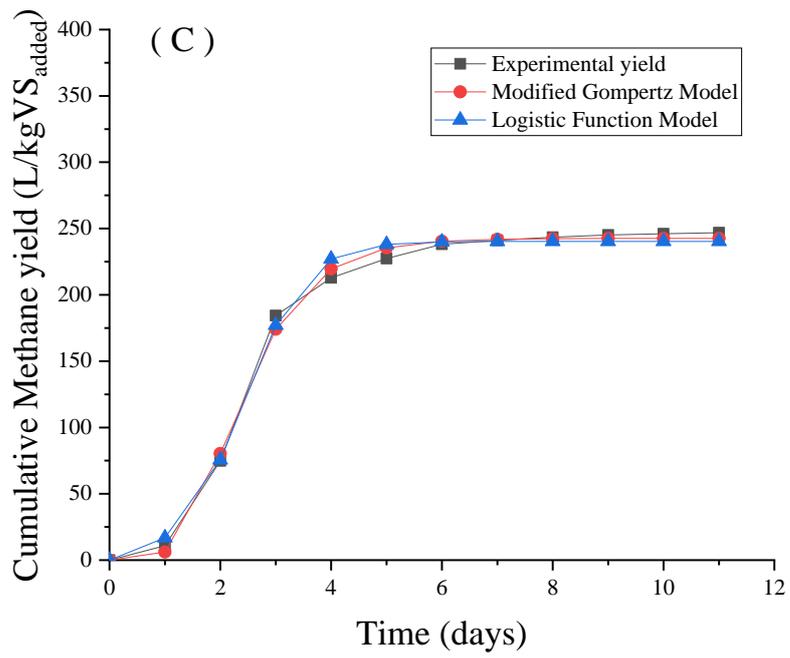
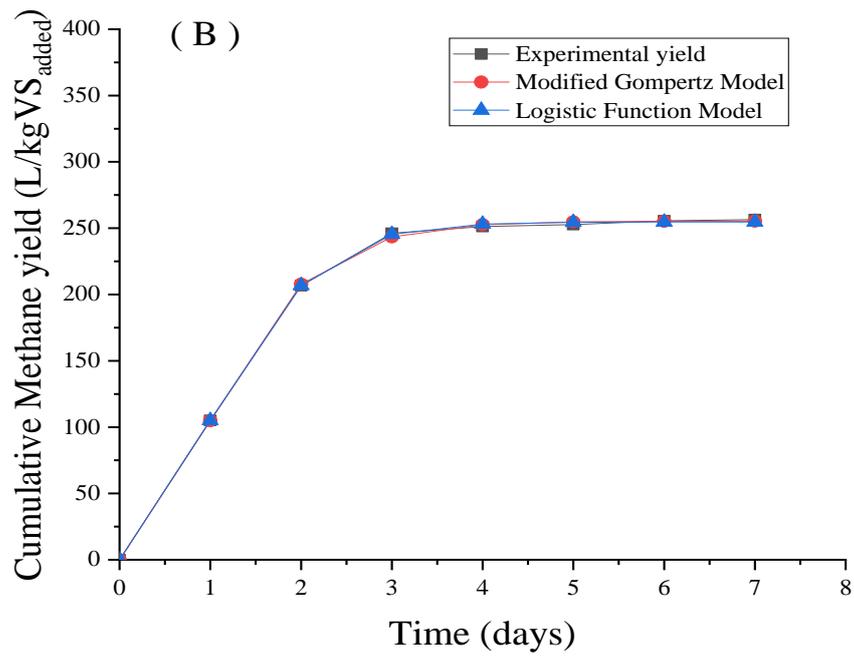
4.1.8 Kinetic modelling for methane gas for anaerobic co-digestion batch study

The simplest model to use for a complex substrate is the first-order kinetic model [Rao MS]. The rate-limiting step in the anaerobic digestion of complex organic materials, such as sludge, is hydrolysis. Applying a first-order kinetic model to calculate the hydrolysis rate constant of organic materials. When OFMSW and bio-flocculated sludge from SST are mixed (75:25), the hydrolysis rate constant is higher than when OFMSW is digested alone. The increased biodegradability of the substrate and the suitable environmental conditions for microorganisms for the entire degradation process are two potential causes of the enhanced hydrolysis rate constant. With the aid of logistic function modelling and curve fitting of the kinetics of the

Modified Gompertz model, the maximum methane production rate (R_m) and lag phase (λ) are found. These models help in the evaluation of the kinetics of methane production [(Ponsa S. et al., 2011). Experimental data are used to develop the Modified Gompertz kinetic model. The experimental results and predicted data are fitted using the Modified Gompertz model with 99% accuracy for various mixing ratios. The Modified Gompertz model demonstrates no significant difference between experimental methane yield and predicted methane yield ($p < 0.05$). The experimental methane yield is supported by this kinetic model.

According to varied mixing ratios in a batch test, presented the results of fitting the kinetic modelling to the anaerobic mono digestion of OFMSW and the anaerobic co-digestion. The figure 13 is created by comparing methane yield experimental data to predictions from the Modified Gompertz model and the Logistic function model for different mixing ratios in the batch study.





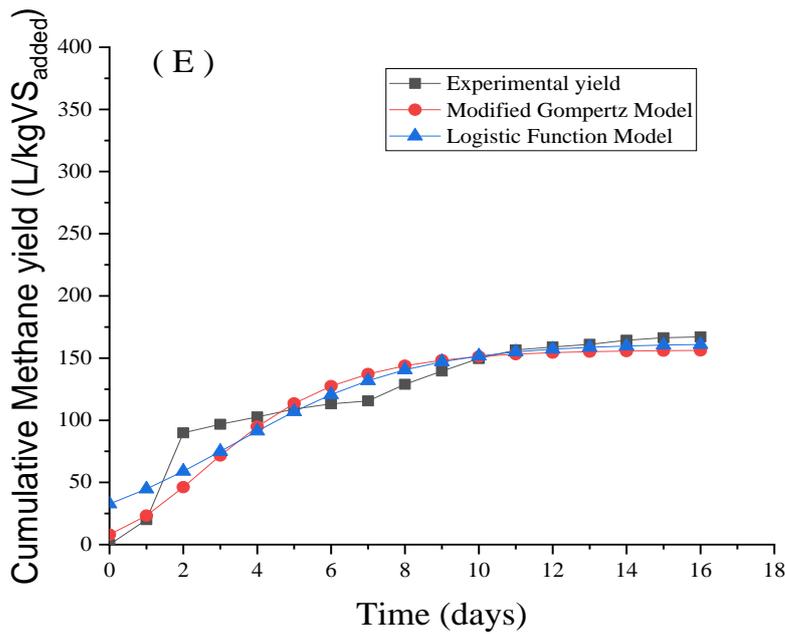
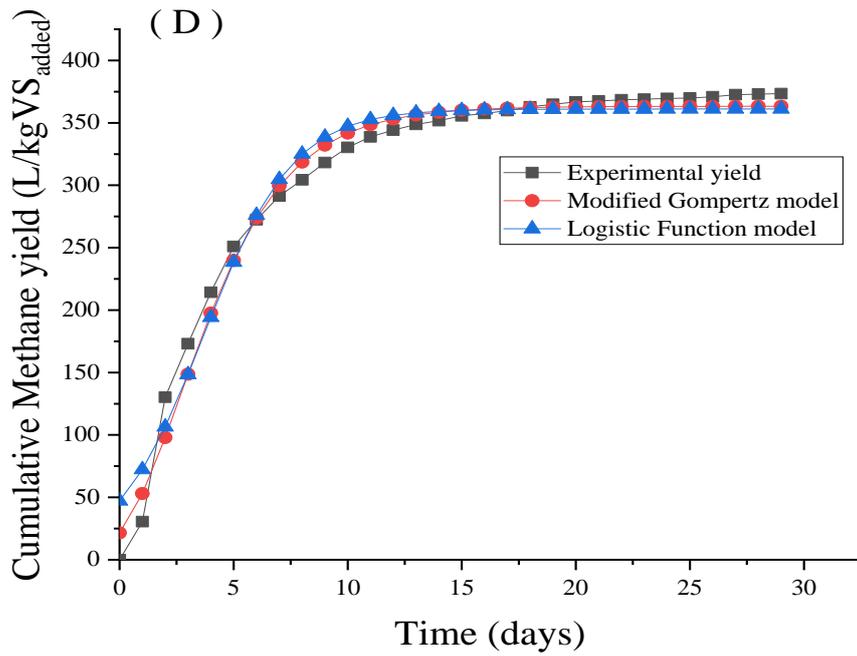


Figure 12: Plot of measured and predicted methane yield using Modified Gompertz model and Logistic function model for different mixing ratios (A) 50:50, (B) 75:25, (C) 90:10, (D) 0:100, (E)100:0

Table 5: Kinetic parameters with different mixing ratios of OFMSW & Bio-flocculated sludge

		Mixing Ratio →				
		50:50	75:25	90:10	0:100	100:0
		Kinetic Parameters ↓				
Hydrolysis rate constant	k	0.226	0.664	0.287	0.211	0.224
	R²	0.9721	0.9833	0.9325	0.9948	0.9507
Modified Gompertz model	P_m	352.06	255.39	242.58	363.28	156.58
	R_m	52.13	137.49	107.35	51.24	25.62
	Λ	0.03	0.24	1.25	0.1	0.2
	R²	0.9277	0.9997	0.9968	0.9848	0.9033
Logistic Function model	P_m	1081.88	763.98	720.92	1083.76	485.51
	R_m	93.99	347.18	325.87	138.70	49.51
	Λ	6.56	1.80	3.04	5.84	6.05
	R²	0.9361	0.9998	0.9949	0.9732	0.9023

Table 5 shows the kinetic parameters achieved using SPSS software shows the hydrolysis rate constant (k) is higher with a mixing ratio of 75:25 with OFMSW and bio-flocculated sludge compared to other co-digestion mixing ratio and mono-digestion of OFMSW. Maximum methane production rate R_m ((L/kg VS_{added}*d) could be achieved with a mixing ratio 75:25 with both the Modified Gompertz Model and Logistic Function model with R^2 0.999 of the study.

4.2 Operational parameters of OFMSW with bio-flocculated sludge using semi-continuous flow anaerobic reactor

The Anaerobic Reactor was operated with different OLRs for a period of time. Availability of OFMSW varies from place to place, season to season and time to time. Considering this real-time application, the composition of OFMSW slightly varied which leads to changes in the characteristics of OFMSW. By changing the composition the % TS of OFMSW is also varied and also affects the COD (mg/gm) and % VS of the feed substrate.

4.2.1 Reactor start-up operation

The anaerobic digestion process is divided into four stages including hydrolysis, acidogenesis, acetogenesis, methanogenesis. To reach the methanogenesis phase, the reactor required

acclimatization time for the growth of bacteria under anaerobic conditions. 10L of acrylic reactor is filled with 7.5kg or 7.35L of OFMSW and bio-flocculated sludge from SST(post-UASB) with a mixing ratio of 75:25 (% wet weight). The reactor is operated for more than 200 days and observed its performance with optimized mixing ratios. The ambient temperature varied from time to time depending on seasonal conditions. Water jacketing is provided to maintain the reactor under mesophilic conditions. Initially, the reactor turns into an acidic condition and takes around 18 days to reach a neutral pH with daily addition of alkali. The day when reactor is started, high production of biogas is observed which contain very less amount of methane gas due to the fermentation process which starts the acidogenesis phase of the anaerobic reactor. After completion of the acclimatization period when pH rises to 6.5 and above, there are chances of methanogens present in the reactor. This biogas production observed on the 18th day with high quantity is rich in methane gas in combination with other gases. Once the methanogenesis phase is achieved, the reactor started feeding with different organic loading rates. Later OLR increased and decreased according to the performance of the anaerobic reactor observed. At the same time, the composition of OFMSW is also varied which led to changes in substrate characteristics. The configuration, alignment and morphological composition of the substrate affect the biogas yield (Hegde and Trabold,2019). The reactor is supplied the feedstock in semi-continuous flow with 8-8-8 hrs or 12-12 hrs or a daily basis. The reactor is provided with a paddle mixer used for intermediate mixing of the substrate. Sample analysis is carried out daily and detailed observation is taken into account. Biogas produced during the anaerobic process is measured with the water displacement method. Biogas is passed through NaOH solution to absorb CO₂ gas generated in biogas.

4.2.2 %VS removal efficiency at different OLR performed with semi-continuous flow reactor

The reactor is operated with the semi-continuous flow with different OLRs to analyse the stability of the reactor with an optimized mixing ratio of 75:25 (%wet wt.) of OFMSW and bio-flocculated sludge from SST (post-UASB). The OLR is systematically varied within the range of 2,3,4,5,6,8 and subsequently 12 gm VS/L/d, considering slight variation and composition and characteristics of substrate reflective of actual field condition.

Figure 14 depicts the observed range of average VS removal efficiency spanning from 84% to 60% throughout the operational performance of the reactor. At OLR 2,3 and 4 gm VS/L/d, VS removal efficiency is observed between 70 to 80%. Contrastingly, with higher OLR average

%VS removal efficiency is observed between 60 to 70% with OLR 5 and 6 gm VS/L/d. Moreover, the VS removal efficiency of about 80% is achieved at OLRs 8 and 12 gm VS/L/d.

During the initial 18 days of time period reactor acclimatizes and after that methanogenesis phase is observed (Figure 19). After the acclimatization period, biogas is generated which is enriched with methane gas. Subsequent operation phases entailed varying OLRs, showing subtle effects on VS removal efficiency. Initial with OLR of 2 and 3 gm VS/L/d, reactor is performed with higher %VS removal efficiency and later increasing OLR of 4 gm VS/L/d leads to decrease in VS removal efficiency and remain between 60 to 70%. Additionally, abrupt OLR increments to 8 and 12 are employed to assess the resilience of the reactor.

Although initially with higher OLR, higher %VS reduction is observed for very short period of time later decreases the pH and increases VFA/Alkalinity ratio creating inhibition due to the accumulation of acids affecting the biogas yield. At higher OLRs of 8 and 12 gm VS/L/d, despite achieving transient high VS removal, the reactor exhibited instability.

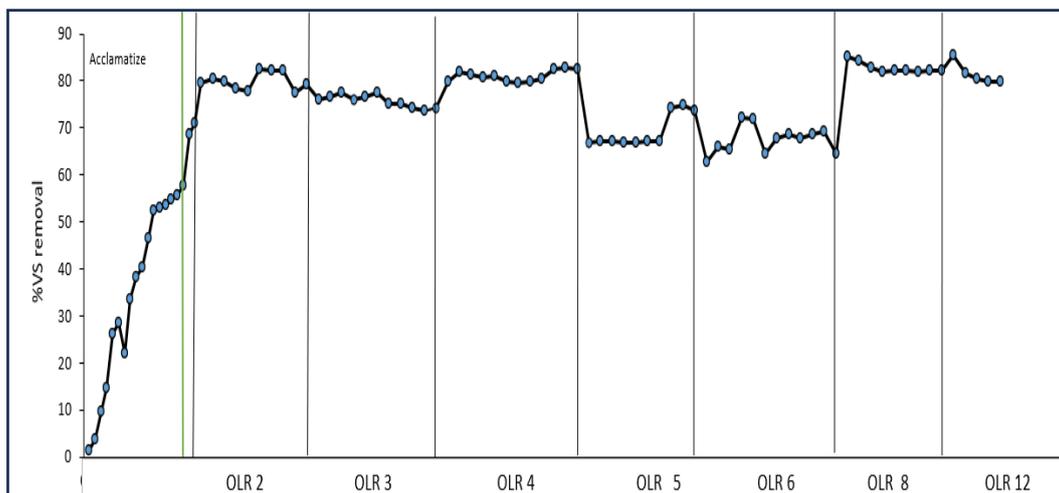
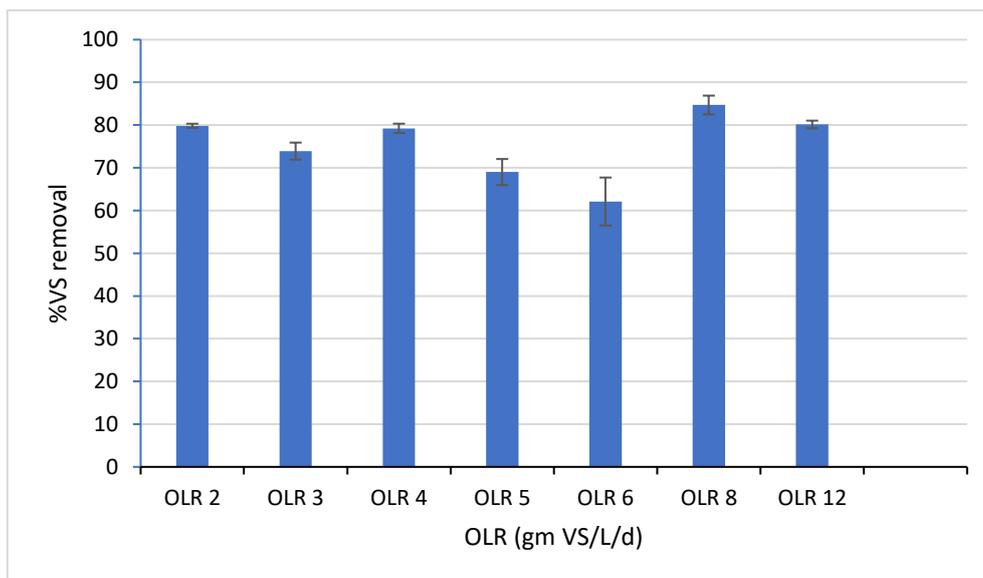


Figure 13: %VS removal efficiency at different OLR

4.2.3 Variation in pH

Figure 15 shows the average pH variation is observed during the operation of a semi-continuous flow anaerobic reactor operated under mesophilic conditions. The pH variation exhibited sensitivity to change in feed OLR, with an average of 6 to 7.5. At a lower OLR feed rate ranging from 2 to 6, the pH remained consistent at 6.5 and above. However, as OLR increased beyond, a disruptive trend is emerged and a decrease in pH 6.5 and below.

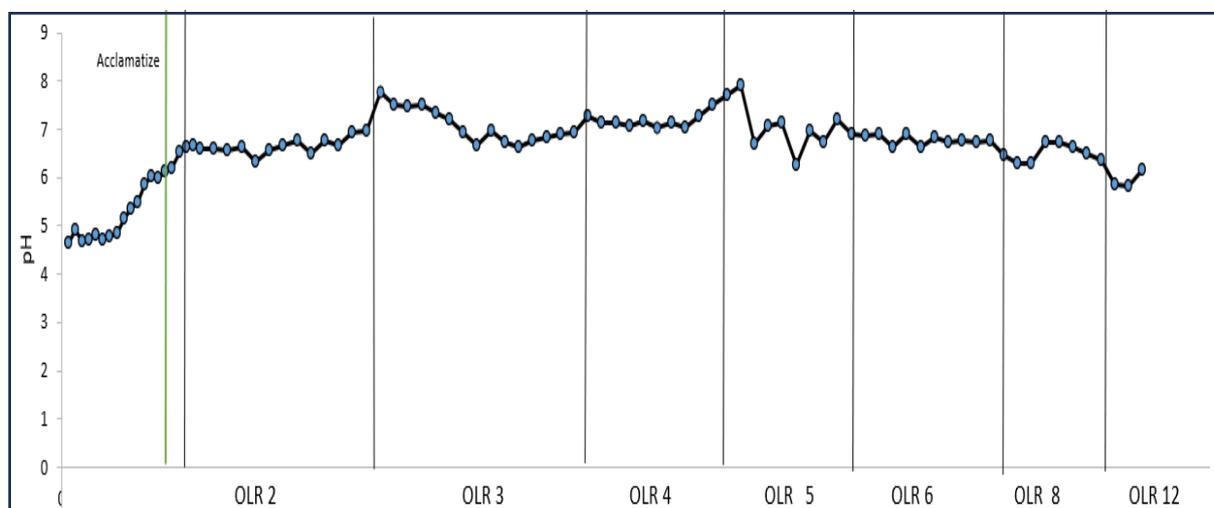
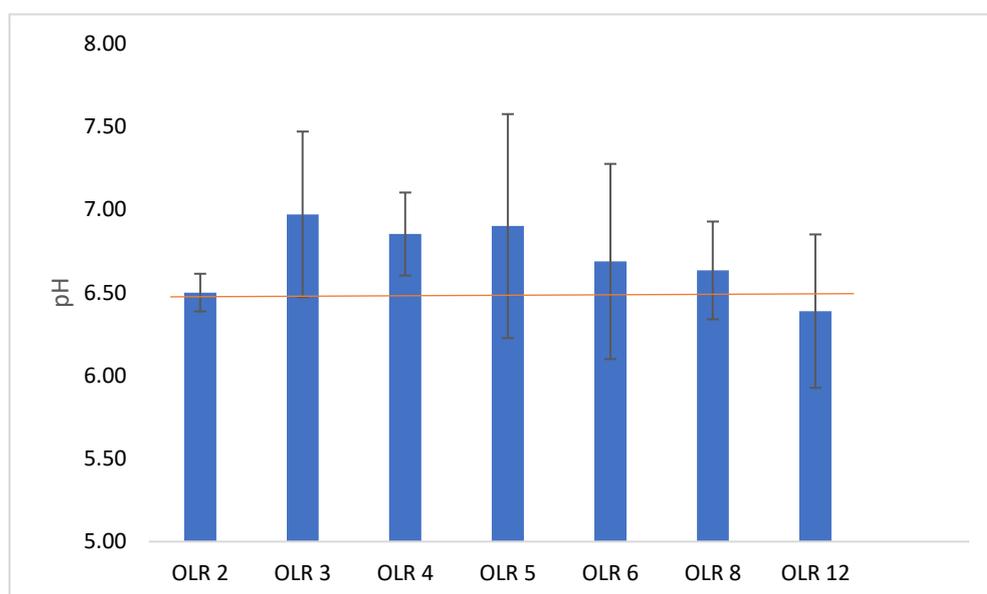
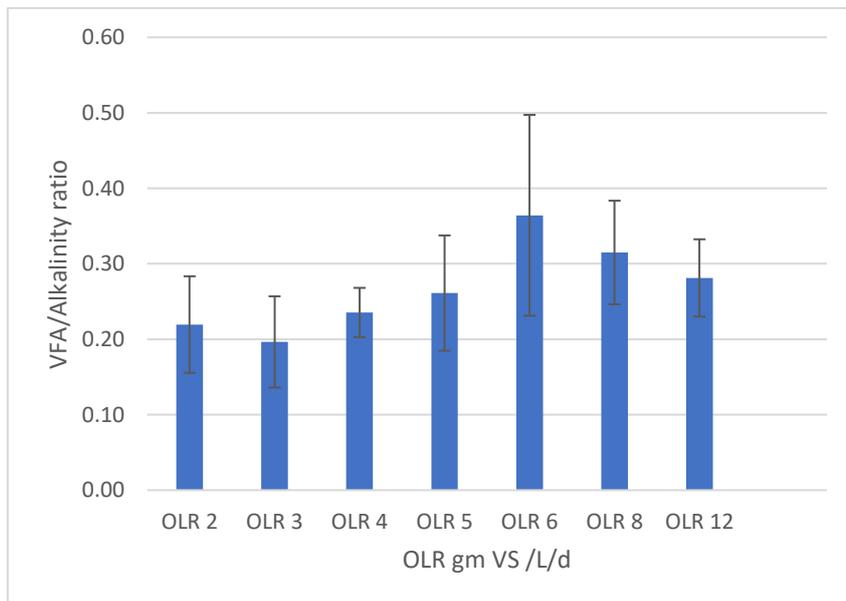


Figure 14: Effect on pH at different OLR

The observed reduction in pH with a higher OLR signifies the accumulation of VFA. This accumulation is leading to a decline in pH creates inhibition within a relatively short time, thereby disturbing the overall performance of the reactor. The increased OLR, reduced pH and the consequential impact on methanogen activation show the importance of maintaining an appropriate pH level for a sustained and efficient anaerobic co-digestion process.

4.2.4 VFA/Alkalinity ratio

VFA is an important indicator of the performance of the anaerobic reactor. It indicates a correlation between the methanogenic bacteria and the breakdown of the Volatile solid. 85% of VFAs are mostly acetate, hence it becomes the most dominating VFA. Analysis of the VFA/Alkalinity ratio provides insight into the digester stability by maintaining a balance between the production rate and consumption rate. Excess production of VFA in the anaerobic digester may reduce the pH which as a result may inactivate the methanogens, hampering the reactor performance.



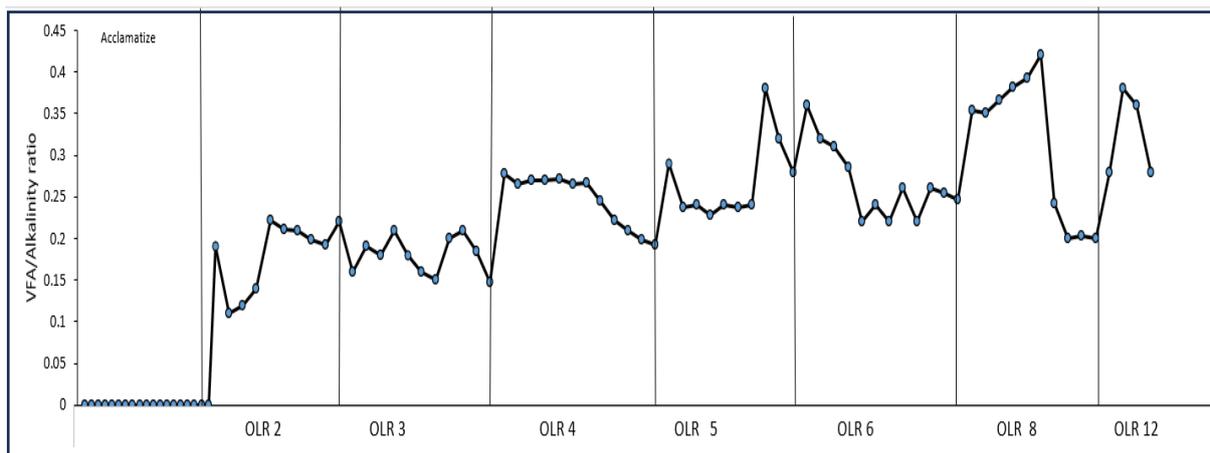
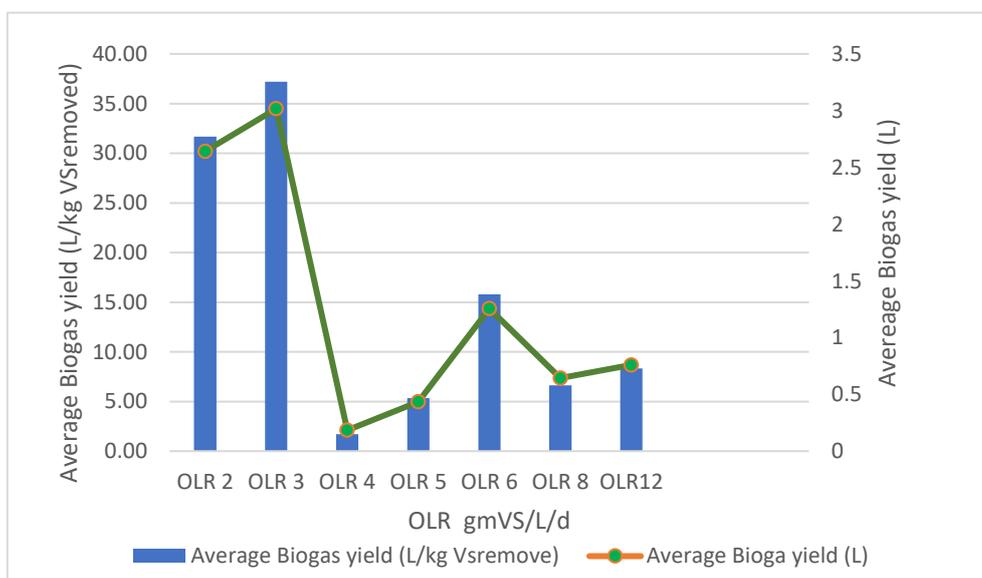


Figure 15 : VFA/Alkalinity ratio at different OLR

Upon introducing various OLRs with different compositions and characteristics of the substrate, the accumulation of fatty acids is noticeable, particularly with an increase in the loading rates. Initially, when the reactor is fed with OLR 2 & 3 gm VS/L/d, the VFA /Alkalinity ratio is observed average 0.2. However, as increasing the OLR, a clear trend emerged indicating elevation in the average VFA/Alkalinity ratio is 0.25 and beyond.

VFA/Alkalinity is considered optimal for the performance of an anaerobic reactor when maintained between 0.1 to 0.2. The observed deviation from the optimal range especially with higher OLRs, shows an imbalance in the anaerobic co-digestion process. This shift in the VFA/Alkalinity ratio may impact the efficiency of the methane gas generation.

4.2.5 Biogas yield with different Loading rates of the substrate



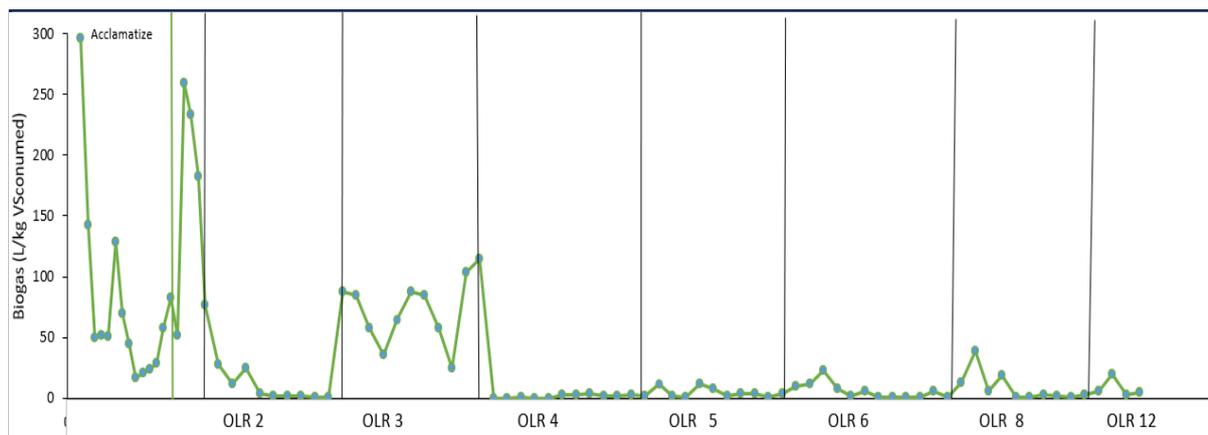


Figure 16: Biogas yield for semi-continuous flow reactor with varied OLR

The biogas yield of semi-continuous flow reactor is observed average 31.68 and 37.20 L/gm $VS_{consumed}$ for OLR 2 & 3 gm VS/L/d respectively (Figure 17). This shows a positive trend with an increase in the loading rate increases the biogas yield.

However, further increasing the OLR, reduces the production of biogas. The decline in biogas yields due to the accumulation of VFA or a decrease in pH both of which can adversely affect the anaerobic co-digestion process. It is essential to note that sometimes with higher OLR biogas is generated with high quantity for a very short period of time but a sudden drop is observed in reactor performance due to increase in VFA/Alkalinity ratio and decrease in pH and also it stops biogas generation. To reboot the reactor operation, the reactor feeds with a lower loading rate. This indicates an anaerobic co-digestion process with varying biogas generation corresponding to different OLRs. The delicate balance required in OLRs for constant biogas production to avoid inhibitory effects of VFAs and pH.

The operation of an anaerobic mesophilic reactor consistently demonstrates fluctuation in biogas yield, pH, VFA/Alkalinity ratio and % VS removal even at the same OLR. This variation is attributed to changes in the characteristics and composition of the substrate utilized for the anaerobic co-digestion process. %TS played an important role in the performance of the anaerobic reactor which is varied for the same OLR and different OLRs which affect the operation condition of the anaerobic reactor. %TS is ranged from 12% to 23% and also once it is fed with 32% Total Solids content as per availability of OFMSW in the field. Throughout the fluctuating %TS range and varying loading rate reactor performs well with a range of OLR 2 to 3 gm VS/L/d. The optimum loading rate for anaerobic reactor performance for OFMSW and bio-flocculated sludge is 3 gm VS/L/d due to a higher biogas yield average of 37.20 L/gm

VS consumed with a lower VFA/Alkalinity ratio of 0.19 ± 0.06 , pH 6.97 ± 0.49 , %VS removal efficiency of 73.91 ± 1.9 during the operation of the reactor. The identified optimal loading rate provides valuable insights for the sustained operation of anaerobic reactors, particularly when OFMSW and bio-flocculated sludge co-digested anaerobically under mesophilic conditions.

4.3 Kinetic modelling study for a semi-continuous flow reactor

4.3.1 Kinetic modelling for cumulative biogas yield for semi-continuous flow anaerobic reactor

The present study uses three popular models namely First-order kinetic model, Modified Gompertz Model and Logistic function model for predicting cumulative biogas yield during the anaerobic co-digestion process (Figure 18). A new hybrid kinetic model that combines the first-order kinetic model and logistic function is developed and applied to experimental data for predicting more accurate cumulative biogas yield with R^2 0.992 compared to other kinetic models. Kinetic models validate the experimental biogas yield. The ability to accurately predict biogas yields is crucial for anaerobic co-digestion processes, enabling efficient waste conversion and maximizing renewable energy production. The findings of this study highlight the modified new model as a valuable tool for enhancing the management of the anaerobic co-digestion process ultimately contributing to sustainable waste-to-energy solutions (Figure 19).

A modified hybrid model is developed to fit the experimental data. Modified hybrid kinetic model (Equation 14) developed with first-order kinetic model and Logistic function model. With the modified kinetic model, we can achieve higher hydrolysis rate constant k with goodness to fit (R^2) 0.992

$$M = P * \frac{(1 - \exp(-k * t))}{(1 + \exp(4 * R * (L - t) / P)) + 1} \quad \text{Eq 14}$$

The equation combines the exponential decay of the first-order model with the logistic function model. The first part of the equation represents the cumulative biogas production that gradually increases over time following exponential growth. The second part of the equation represents the logistic function model which introduces a sigmoidal growth curve for biogas production. By combining the First-Order and Logistic Function models, this equation provides a more comprehensive representation of the kinetics of anaerobic digestion, capturing both the initial exponential growth and the subsequent levelling-off as the system approaches its maximum biogas production potential. Kinetic parameters k , P , L , and R were determined using

experimental data. this equation assumes certain underlying assumptions about the anaerobic digestion process, and its applicability may vary depending on the specific conditions and characteristics of the system being modelled.

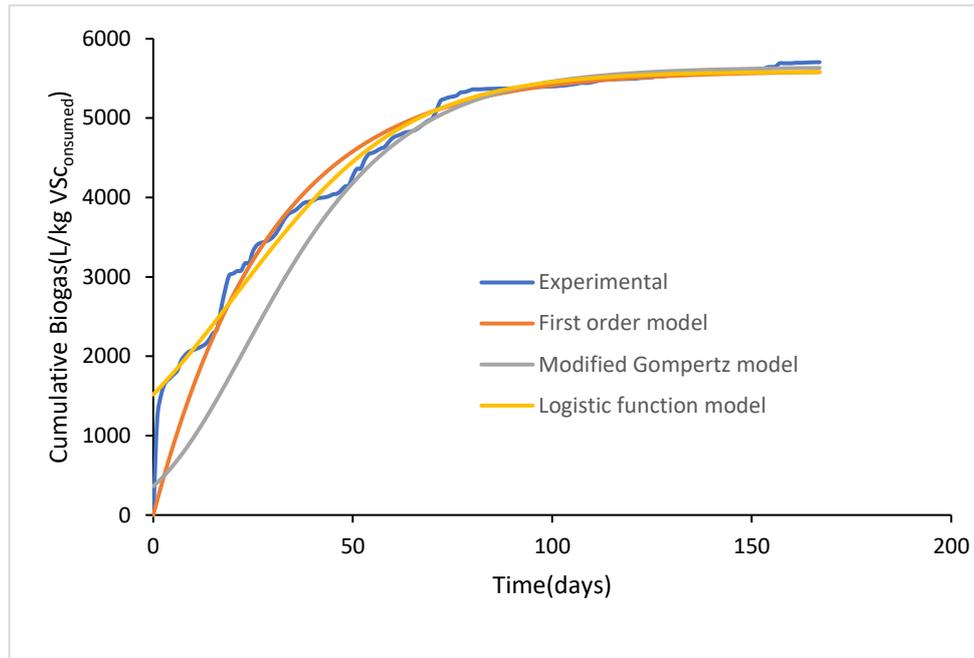


Figure 17 : Kinetic Models -Modified Gompertz Model, first-order kinetic model, Logistic Function model for cumulative biogas yield

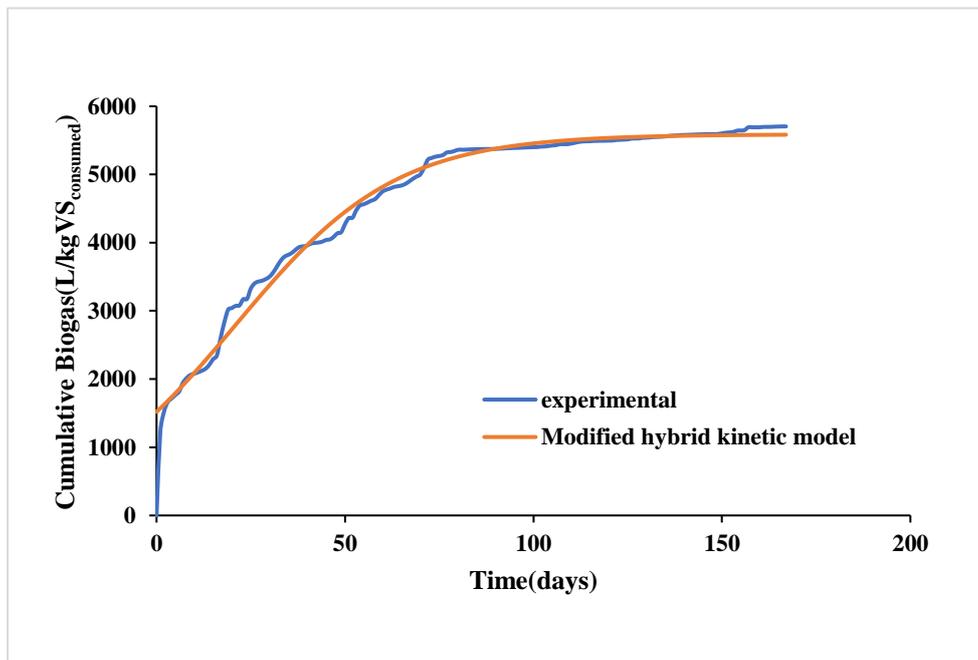


Figure 18: Modified hybrid kinetic model for cumulative biogas yield for semi-continuous flow anaerobic reactor

Table 6 depicts the Kinetic constants are developed for anaerobic co-digestion semi-continuous flow reactor where both the model shows higher accuracy while the modified new model shows also a higher hydrolysis rate constant.

Table 6: Kinetic constant for semi-continuous flow anaerobic co- digestion

Kinetic parameters	First-order kinetic model	Modified Gompertz model	Logistic Function model	Modified hybrid kinetic model
k	0.034	-	-	0.689
P _m	5598	5642	16765	11225
R _m	-	92	197	123
Λ	-	0.2	44	36
R ²	0.974	0.98	0.983	0.992

4.3.2 Kinetic modelling for substrate removal efficiency

Three popular kinetic models The Modified Stover Kincannon model, Grau's Second Order model and First Order kinetic model are chosen to find kinetic constants for substrate removal efficiency for anaerobic co-digestion process.

4.3.2.1 Modified Stover-Kincannon model

U_{max} provides an estimate of the maximum rate at which organic matter is converted to biogas in an anaerobic digester. K_B reflects the threshold beyond which the substrate concentration starts to saturate the system and inhibits further removal. By knowing U_{max} and K_B, we can make decisions about factors like the OLR, to achieve the desired COD removal rates and biogas production. Figure 20(A) shows the developed Modified Stover-Kincannon model. Here Equation 15 shows developed Modified Stover-Kincannon model achieves a Substrate removal rate efficiency with R² 0.8619

$$Se = Si - \frac{31.74 * Si}{86.02 + (Q * \frac{Si}{V})} \quad \text{Eq. (15)}$$

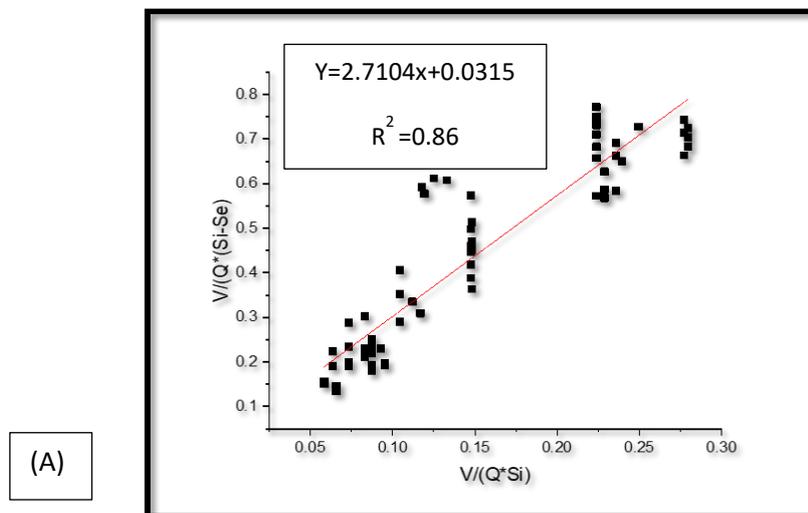
4.3.2.2 Grau's Second Order model

The plots of HRT vs. HRT/COD_{removal} to get the Grau's Second Order constant a and b, are shown in Figure 20(B). A strong correlation coefficient ($R^2 = 0.9002$) is found by looking at the best-fitted line's intercept and gradient. These values, i.e. for a and b, can be used to predict process efficiency, with the COD effluent concentration (S_e) from Anaerobic co-digestion. A high "b" value indicates that the process can be prone to inhibition, and steps like substrate pretreatment or dilution might be done to lessen inhibition. Eq. (16) predicts the COD content in the effluent substrate for anaerobic co-digestion process.

$$S_e = S_i * \left(1 - \frac{HRT}{0.578 + 3.1 * HRT}\right) \quad \text{Eq. (16)}$$

4.3.2.3 First-Order kinetic model

The generated First-Order kinetic model is shown in Figure 20 (C). The First Order rate constant k_r 0.12 is obtained with R^2 0.8018. A higher k_r value indicates a faster rate of COD removal which may be desirable for quicker waste degradation and biogas production. the low value of the correlation coefficient (0.80) indicates that the First-Order substrate removal model cannot be applied with a good degree of precision.



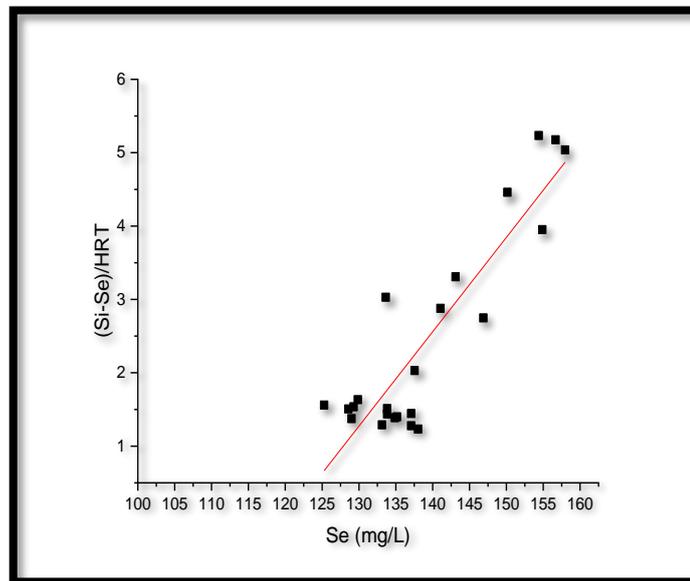
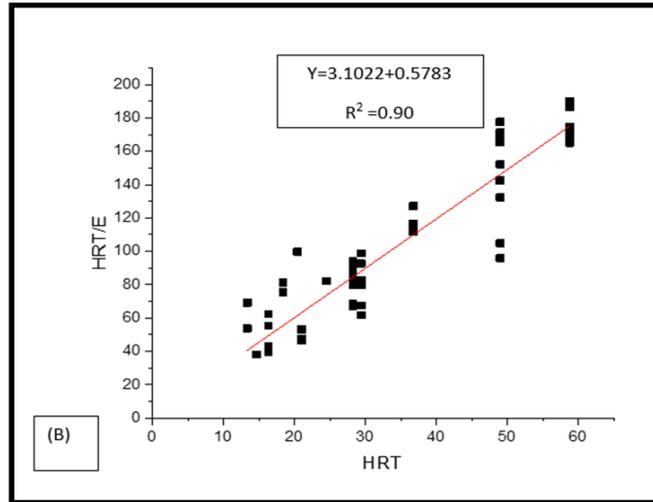


Figure 19 : Plot for evaluation of kinetic model (A) Modified Stover Kincannon model (B) Grau's second order kinetic model (C) First order kinetic model

Table 7 depicts the comparative analysis of various kinetic constants found in present study with different literature.

Table 7: Comparative study of kinetic constants for substrate removal

Substrate	Reactor type	Kinetic constants					References
		Modified Stover Kincannon		Grau's Second order		First order	
		K_B	U_{max}	a	b	k_r	

OFMSW & bio-flocculated sludge from SST	Anaerobic mesophilic semi-continuous flow reactor	81.86	37.45	0.578	3.1	0.12	Present study
Cannery Seafood	UASB	15.34	15.47	0.217	1.009	-	(Jijai et al., 2016)
Piggery waste	MBBR	52.40	82.65	-	-	-	(Nguyen et al., 2021)
Tannery wastewater	ASBR	5.56	5.78	0.87	1.01	0.99	(Andualem et al., 2017)
Oily wastewater from petroleum refinery	MBR	6.88	6.41	0.152	0.945	1.26	(Ahmadi et al., 2019)

4.3.2.4 Kinetic modeling for prediction of effluent Volatile Solids with Modified Stover Kin-cannon Model and Grau’s Second-order Kinetic model:

4.3.2.4.1 Modified Stover Kin-Cannon model for prediction of Volatile Solids in effluent

Kinetic model Modified Stover Kin-cannon applied for prediction substrate removal on Volatile solids basis. Biogas generation is directly dependent on the degree of degradation of VS. Prediction of VS in effluent is an important parameter to understand the anaerobic reactor performance. Modified Stover Kin-cannon model is applied for semi-continuous flow anaerobic digestion process to calculate kinetic constant for VS in effluent. The kinetic constant achieved with effluent VS, U_{max} & K_B are 72.46 and 130.43 respectively with R^2 0.9335 (Figure 21). Figure 22 shows predicted and experimental volatile solids present in the effluent sample after anaerobic digestion.

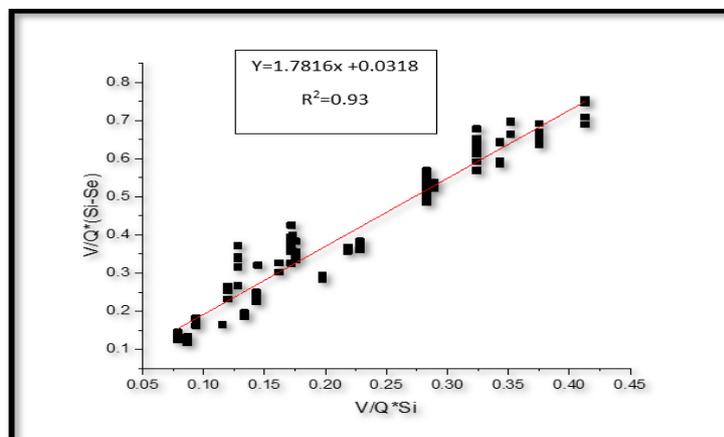


Figure 20: Modified Stover Kin-Cannon Model for output of Volatile Solids (gm/L)

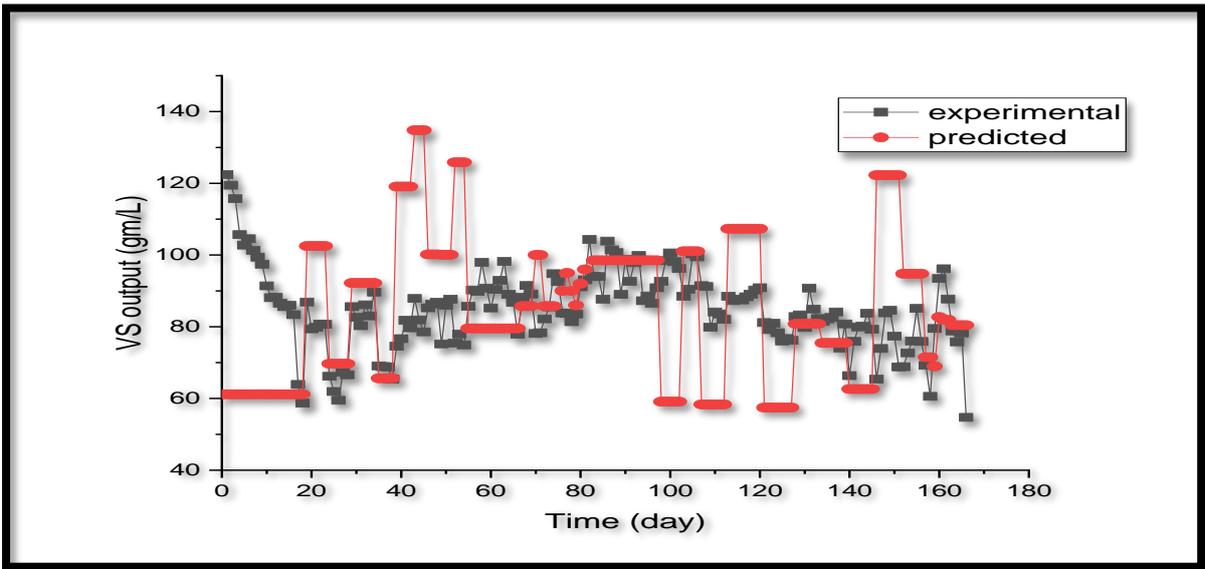


Figure 21: Experimental and Prediction of Volatile solids output (gm/L) with Modified Stover Kin-Cannon model

4.3.2.4.2 Grau's Second Order kinetic model

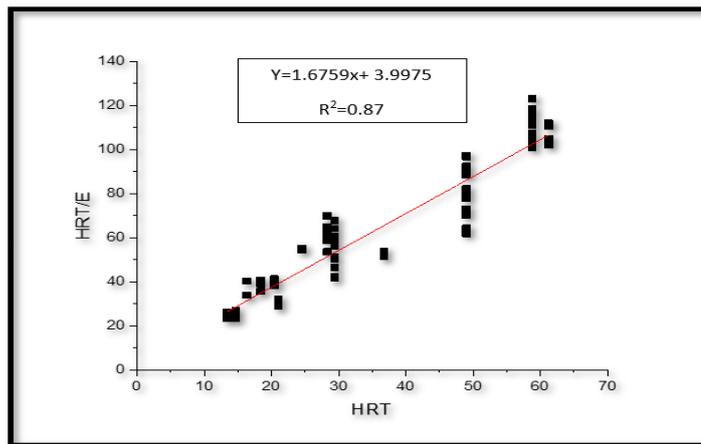


Figure 22: Grau's Second-order kinetic model for Volatile Solids output

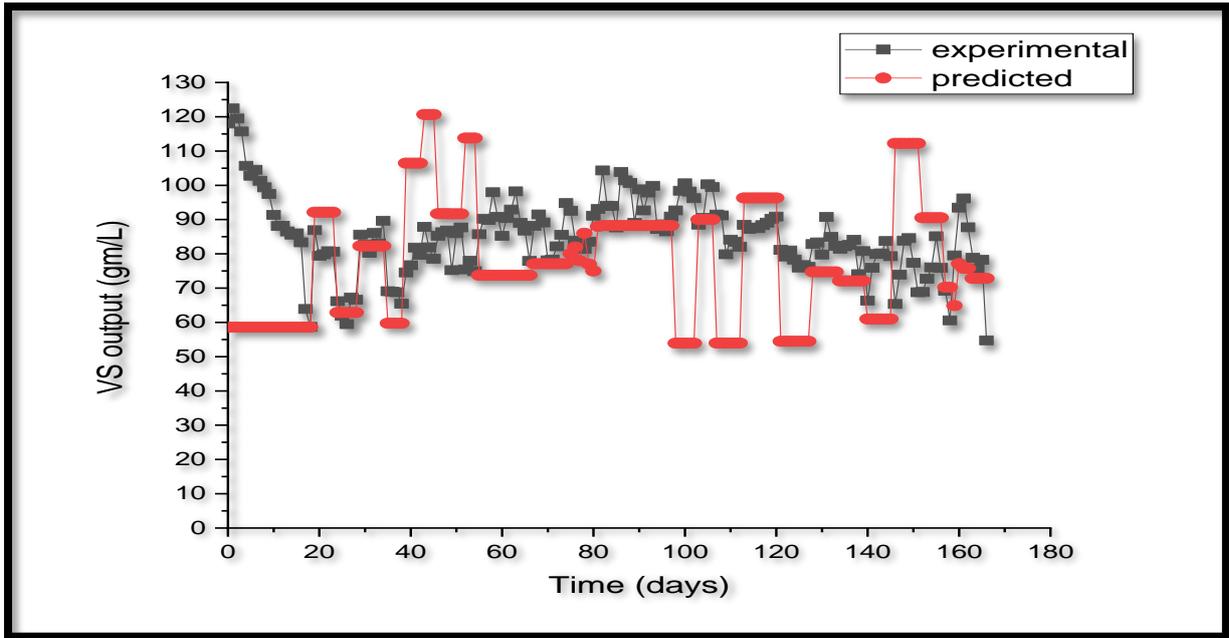


Figure 23: Experimental and predicted Volatile Solids output with Garu's second-order equation

Grau's second-order kinetic model was also applied for effluent volatile solids prediction and kinetic constants a and b were 3.99 and 1.67 respectively with R^2 0.870. Kinetic constants a, b are used to determine predicted values of effluent VS concentration as seen in Figures 24 which shows the relationship between predicted and experimental volatile solids concentration.

4.4 Prediction modelling using Artificial Neural Network (ANN)

Prediction modelling is a very important tool for the performance of an anaerobic digester. In the real-time application of an anaerobic co-digestion process, the composition and characteristics of OFMSW and bio-flocculated sludge from SST will vary from time to time and season to season. Large variation was observed for the composition of OFMSW due to seasonal conditions. Developing the prediction model with existing lab-scale experimental data is a necessity to replicate this process for field operational conditions. Using the prediction model operator can control different parameters to achieve an uninterrupted anaerobic co-digestion process. To develop a prediction model using ANN total of 102 days of data was utilized from lab scale semi-continuous flow anaerobic co-digestion process when methane gas production was observed with different OLR.

Input variables taken into consideration were %TS, OLR (gmVS/L/d), pH, VFA/Alkalinity ratio, HRT(days) for the development of the prediction model with output variable Methane yield (L/kgVS_{removed}) and % VS_{removed}. % Total Solids (TS) represent the total mass of solids in

a given influent substrate. It includes both organic and inorganic solids. TS does not distinguish between the organic fraction and the inorganic fraction. Organic fraction contributes to methane generation. The TS content of the feed substrate changes from 12% to 32% in the current semi-continuous feed anaerobic co-digestion study. The reactor was operated with a substrate with high total solids. Organic matter present in the substrate required to measure is the most significant aspect of solid analysis. The OLR is the most crucial parameter for the effective operation of the anaerobic reactor. OLR ranges in this study varied between 2 to 12 gmVS/L/d. The amount of organic matter added to the anaerobic digester per unit of reactor volume and time is measured by the OLR. Grams of VS per litre of reactor volume per day (gm VS/L/d) is the standard unit for OLR measurements. It conveys the rate at which the system is supplied with organic matter. The phases of the anaerobic co-digestion process are measured with pH. A sudden drop in pH is observed during fermentation with active acidogenesis bacteria. The methanogenesis phase is achieved when pH stabilized between 6.2 to 8.36. VFA concentration must be managed in the reactor for optimal treatment efficiency and methane yield. VFA concentration in the system immediately affects COD removal. The ability of the substrate to neutralize acids is known as its alkalinity in the digester. VFA/Alkalinity ratio offers a CO₂ buffering capability for methane generation in addition to pH management. In this study, the VFA/Alkalinity ratio ranges from 0.1 to 0.54 during the study period. In the present study, the experiment is performed for semi-continuous flow only and HRTs are ranged between 15 to 45 days (mean 34 days, maximum up to 74 days) in the overall study of more than 200 days. The efficiency of any anaerobic digestion process is typically observed with the production of biogas or methane gas. Maximizing the biogas yield rate as a result of the biodegradation of the organic part of the waste is a crucial step to take into account for the operation of the reactor. This can be achieved with close monitoring of the anaerobic co-digestion process. Reduction in methane yield is the indication of unstable reactor condition. The rigorous supervision of the biogas (both visual/on-site and computer-based) helps assure the stability of these vulnerable systems. Before being disposed of in the environment, the anaerobic co-digestion process helps to lessen the pollutant load. 55.5% to 87 % of the VS are reduced over the research period.

4.4.1 Artificial Neural Network-based prediction model using fitting application

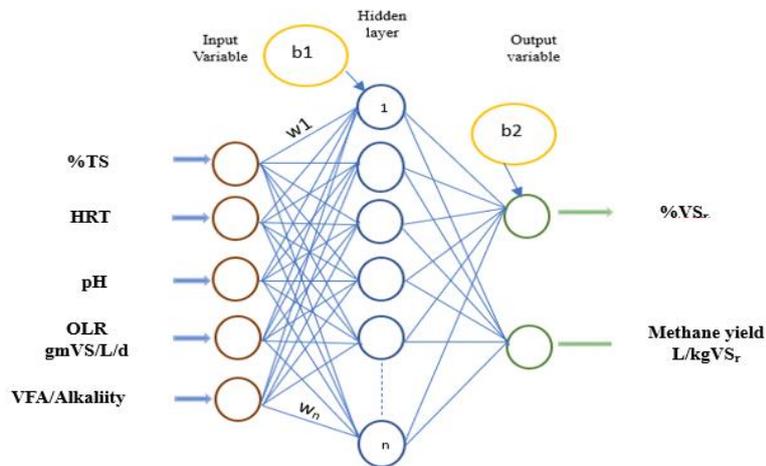


Figure 24 : Architecture of Artificial Neural Network

A fully connected feed-forward neural (fitnet) network is utilized for this investigation. The input layer has five input variables, one hidden layer has n hidden neurons, and one output layer has two output variables. A bias neuron is also present in the input and hidden layers, and it provides each neuron in those layers with constant activity.

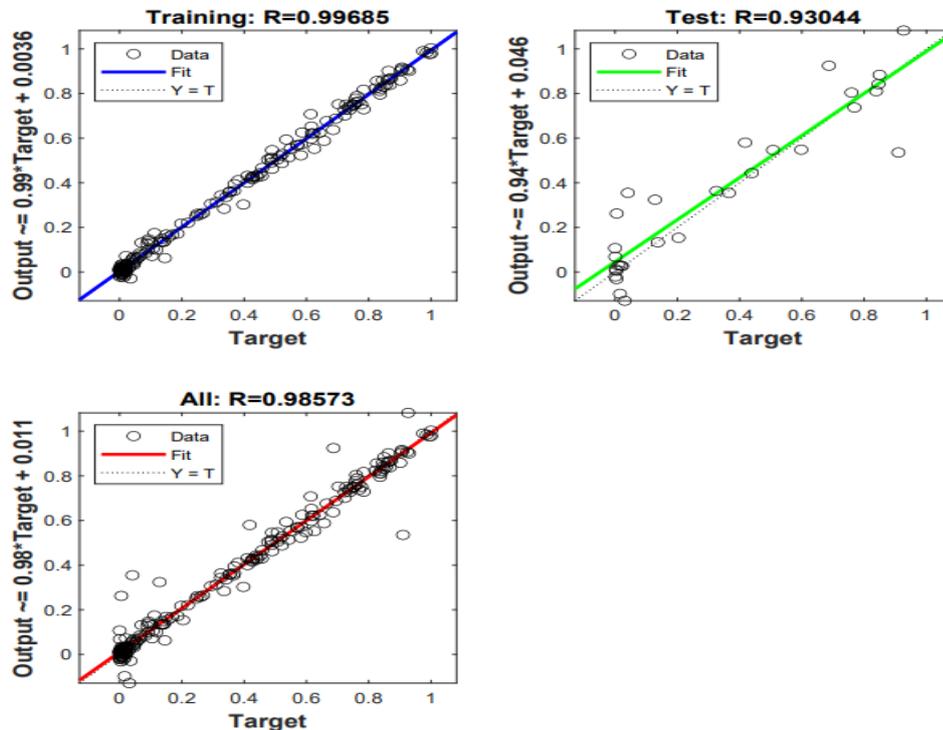


Figure 25 : ANN performance model with training function Bayesian Regularization

The ANN model utilized the sigmoidal activation function (Olden & Jackson, 2002). As per equations (17) and (20) The suggested ANN architecture has been assessed based on the Mean

Squared Error (MSE) and correlation coefficient (R). Figure 26 shows a graphic representation of the neural network performance using a regression curve and the best validation performance curve for training function Bayesian Regularization. According to equations (18) and (19), the mean absolute error (MAE) and mean absolute percentage error (MAPE) are computed.

$$\text{Mean Squared Error (E)} = \frac{1}{x} \sum_{i=1}^x (P_i - E_i)^2 \quad \text{Eq. 17}$$

$$\text{Mean Absolute Error (MAE)} = \frac{1}{x} \sum_{i=1}^x |E_i - P_i| \quad \text{Eq. 18}$$

$$\text{Mean Absolute Percentage Error (MAPE)} = \frac{1}{x} \sum_{i=1}^x \left| \frac{E_i - P_i}{E_i} \right| \times 100\% \quad \text{Eq. 19}$$

$$R = \frac{\sum_{i=1}^x (P_i - \bar{P}_i)(E_i - \bar{E}_i)}{[\sqrt{\sum_{i=1}^x (E_i - \bar{E}_i)^2}][\sqrt{\sum_{i=1}^x (P_i - \bar{P}_i)^2}]} \quad \text{Eq. 20}$$

Where,

x = number of datasets, E_i = experimental data, \bar{E}_i = mean of experimental data, P_i = Predicted data output, \bar{P}_i = mean of predicted data output, R = coefficient of correlation

The lowest MAE, MAPE, and MSE values and R of several training techniques were used at various numbers of hidden neurons for a trained ANN model for %VS_{removal} and Methane(L/kgVS_{removed}) response. The Feed Forward Neural Network with Bayesian Regularisation (BR) technique is the training strategy that provides the best R -value (0.986) for the %VS_{removal} processes at 17 hidden neurons, the lowest MAE value of 0.419 and the lowest MAPE value of 0.006 and lowest MSE value of 0.697. Furthermore, from two neurons to twenty neurons, the MAPE error has never surpassed 10% while employing the BR training procedure. Using the BR training technique and 19 hidden neurons, the greatest R -value of the neural network after training is 0.97 for methane yield. It is interesting to note that the BR and SCG training algorithms produced the greatest and lowest MAE values at 19 hidden neurons and 2 hidden neurons, the BR training approach outperforms others in terms of MAPE performance criterion. Once more, BR and LM trained the most effective ANN model when compared to those who had the lowest MAE, MAPE, and MAE values. The feedforward neural network with tan-sigmoid (fitnet) is trained using the Bayesian regularisation (BR) approach. Neural Network architecture 5-19-2 shows the correlation between experimental and predicted data for methane yield with $R^2 = 0.9533$ in Figure 27 whereas %VS_{removal} with network architecture 5-17-2 shows $R^2 = 0.9782$ for the experimental and predicted data in Figure 28.

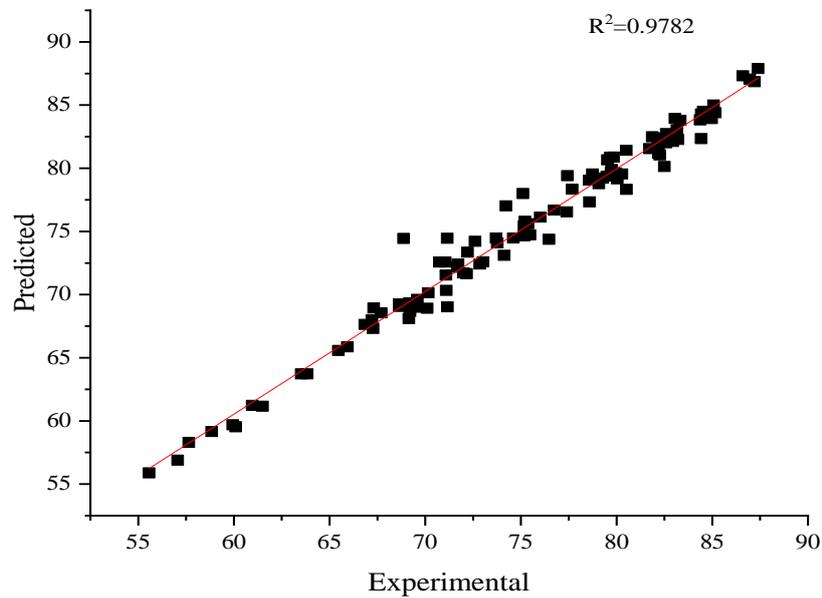


Figure 26: Correlation between experimental data and predicted data of Methane yield (L/kgVSremoved)

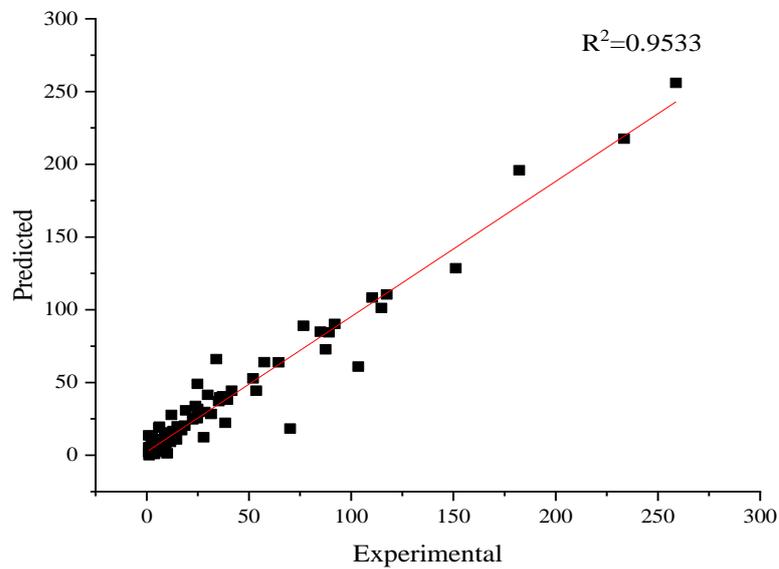


Figure 27 : Correlation between experimental data and predicted data of % VS_{removed}

4.4.2 Relative Importance of input variable for anaerobic co-digestion process

Here, we summarise the significance of input and output variables for a feed-forward backpropagation neural network with training function BR and tan-sigmoid activation function

for 14 hidden neurons. It demonstrates that % VS_{removal} and Methane yield for semi-continuous flow Anaerobic Reactor is significantly concerned with %TS of the input substrate, followed by HRT and Organic Loading Rate. The factors that affect Methane yield are %TS > pH > VFA/Alkalinity ratio > OLR (gmVS/L/d) > HRT.

Table 8: Relative Importance of input variable using connection weight approach

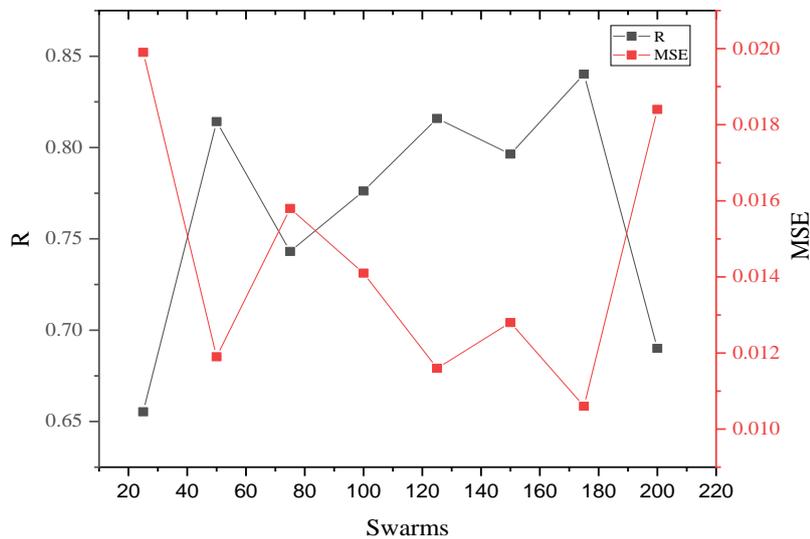
Output variable	Input variable	Connection weight	Rank
% VS _{reduction}	%TS	-12.29409533	1
	OLR (gmVS/L/d)	-7.579649335	3
	HRT	10.32815392	2
	VFA/Alk	1.007274868	5
	pH	-1.109764449	4
Methane yield(L/kgVS _{removed})	%TS	13.84728014	1
	OLR (gmVS/L/d)	-3.922425271	4
	HRT	3.40389466	5
	VFA/Alk	6.268103061	3
	pH	-7.293040643	2

4.4.3 Prediction modelling using ANN-PSO

In this study, the feed-forward neural network is contrasted with the robust stochastic optimization method known as Particle Swarm Optimisation (PSO). In the ANN-PSO model, PSO is used to reduce the errors of the ANN by updating the best weights and biases for the model. As a result, the weights and biases in this issue are the variables, and the range of these variables' variations determines how feasible the task is. The fitness function used in this study is a Mean Squared error, which is calculated by dividing the total number of input and output datasets by the sum of the squares representing network inputs and outputs (Alam, 2016). When it comes to improving the training process, parameter selection is essential. The convergence rate may be greatly influenced by a single parameter. PSO has been used in this work to optimize the input parameters with updating weight and bias generated with FFNN. PSO has been used with five input parameters and one output parameter where upper boundaries and

lower boundaries are set for the optimization of parameters. The inertia weight controls the impact previous velocity of particles on their current velocity which ranges between 0.1 to 0.5 during the study. When using the PSO algorithm, some PSO parameters like C1 and C2 need to be supplied. C1 represents how much each particle is influenced by its own best position while C2 represents the social learning rate which determines how much a particle is influenced by its gbest position. As a result, the suggested ANN-PSO framework has been performed several times with various parameter values. According to the results of the many runs, the values of C1 and C2 should be 2.67 and 1.34, respectively, to obtain the best fitness and quick convergence with PSO. Figure 29 (B) shows how the MSE varies depending on swarm size and number of neurons. To track the MSE fluctuation, a total of 2000 iterations have been completed. The MSE was 0.0817 in the first iteration and reduced to 0.0106 after 2000 iterations. The regression plot has also been investigated for 18 neurons and 175 swarms to evaluate the performance of the ANN-PSO algorithm. The suggested ANN-PSO regression curve and convergence are shown in Figure 30. The ANN-PSO correlation coefficient is 0.84015. The computed R-value shows that the methane generated from the experimental ACoD of OFMSW with bio-flocculated sludge from SST is near the projected value of the biogas from the ANN-PSO algorithm.

(A)



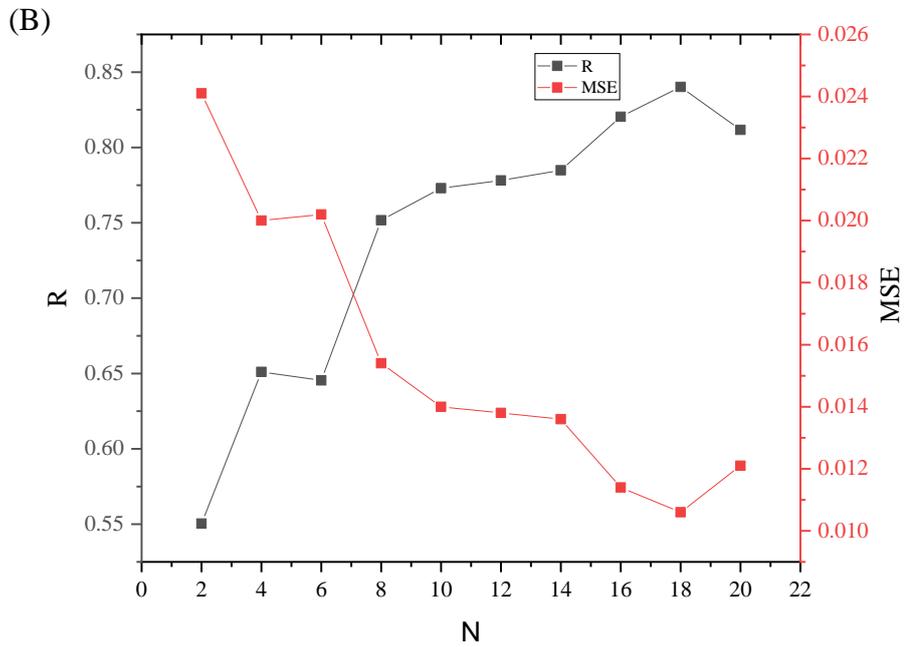


Figure 28 : (A) ANN-PSO model performance of anaerobic co-digestion for different numbers of Swarm size

(B) ANN-PSO model performance of anaerobic co-digestion for different numbers of Neurons

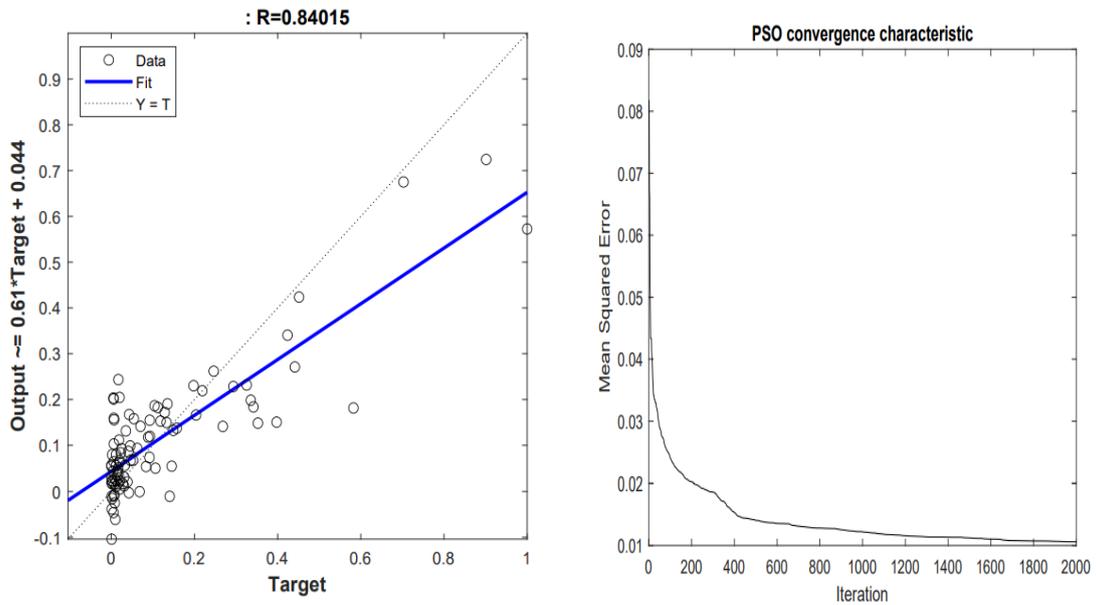


Figure 29: ANN-PSO (A) regression (B)convergence

Figure 31 shows experimental and predicted methane yield using ANN-PSO with R^2 0.8058. The model accuracy shows that this model can help predict accurate methane yield for unknown input variables.

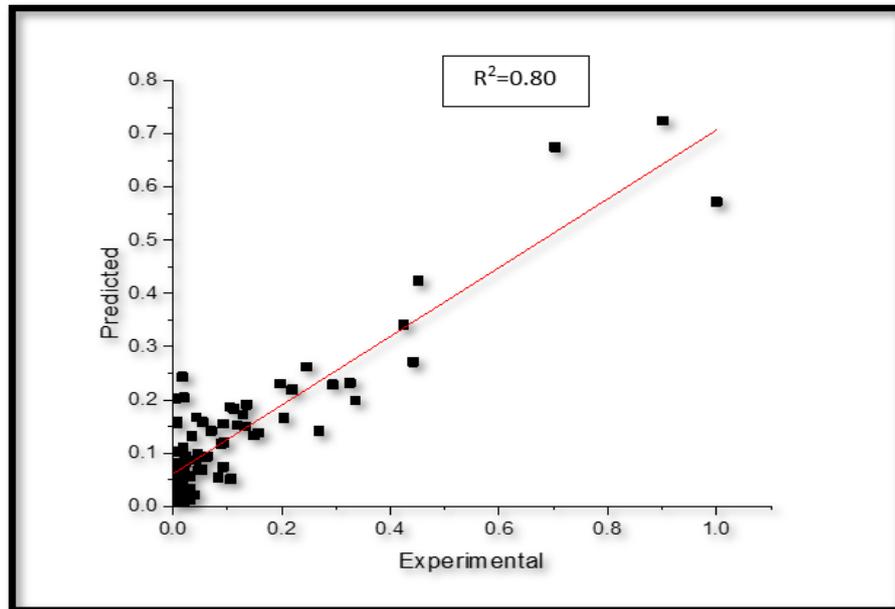


Figure 30: Experimental and Predicted Methane yield using ANN-PSO

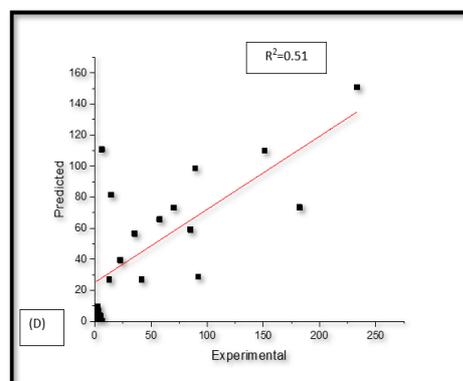
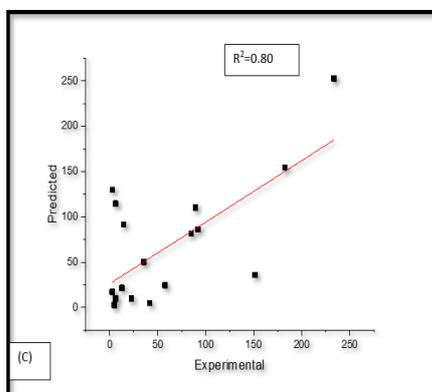
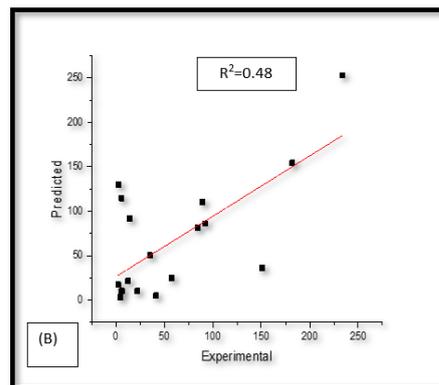
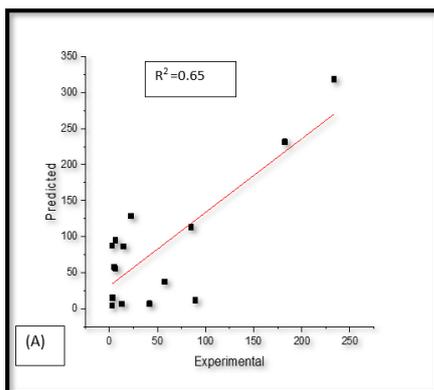
4.4.4 Prediction model using ANFIS

A data share of 75% is used to train the model, and the other 25% is used to test the developed model. The initial ANFIS network is trained with five different types of MFs triangular shaped, Trapezoidal type, Gaussian type, Generalized bell shaped, Gauss2mf type. The results of each membership function are tabulated in Table 9 by using the MAPE and R^2 values as the value of network performance. Based on the results Gauss2mf type membership function with minimum MAPE value has the minimum error between predicted and experimental values and the maximum R^2 value can export more accurate results compared to the other four types of membership functions. After the training process, the ANFIS models are tested using an independent data set. The outputs of the network are extracted and the performance of the network is calculated. As a result, the Gauss2mf type membership function is a maximum R^2 0.90 value for experimental testing data, and predicted test data is considered the best membership function for the development of a prediction model of OFMSW and bio-flocculated sludge using ANFIS. Figure 32 shows model accuracy using experimental and predicted data with different membership functions. Gauss2mf (gaussian-2 membership function) has two adjustable parameters (i) center and (ii) spread. The Centre determines the center of a bell-shaped curve and the spread controls the width of the curve. Adjusting these

two parameters gauss2 membership function fits the training and testing data. Gauss2mf is flexible and can effectively deal with complex, non-linear relationships. Gauss2mf can provide a good fit between input and output variables and has gaussian like distribution.

Table 9: Development of ANFIS prediction model using different membership functions for methane yield

Membership function		Triangular shaped	Trapezoidal-shaped	Gaussian type	Generalized bell-shaped	Gauss2mf type
MAPE	Training	0.055	0.092784	0.085241	0.017223	0.030273
	Testing	5.464837	4.707699	2.156461	7.055921	2.448057
	All	1.436357	1.271061	0.614064	1.814337	0.647579
R^2	Testing	0.65	0.47	0.79	0.51	0.90



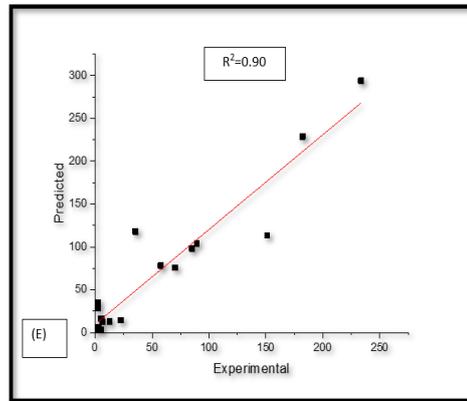


Figure 31: Co-relation between experimental and predicted ANFIS model data using different membership functions for methane yield using (A)trimf, (B)trapmf, (C) gaussmf, (D)gbell, (E)gauss2mf

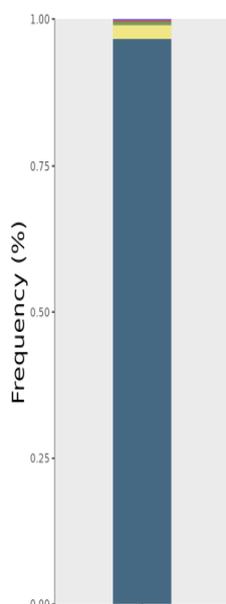
4.5 Metagenomic Analysis

It is important to understand the microbial consortia in terms of taxonomy, diversity and metabolic pathways. The studies on metagenomics of micro-organisms involved in anaerobic digestion have gained momentum with advancements in sequencing methods. This understanding helps in the synergistic relationship between micro-organisms and digester performance. It also helps to improve process economic viability of anaerobic digestion and to maximize the methane yield. It also helps to analyse the metabolic pathway which is important to engineer the environmental parameters to augment the development and action of key genera. Microbial diversity in Anaerobic Digestion depends upon various factors like substrate, pretreatment, mixing, temperature, OLR etc. However, very little research is carried out on microbial growth during fermentation. Amplicon sequencing methods targeting the 16S rRNA gene have been used extensively to investigate microbial community composition and dynamics in anaerobic digestion.

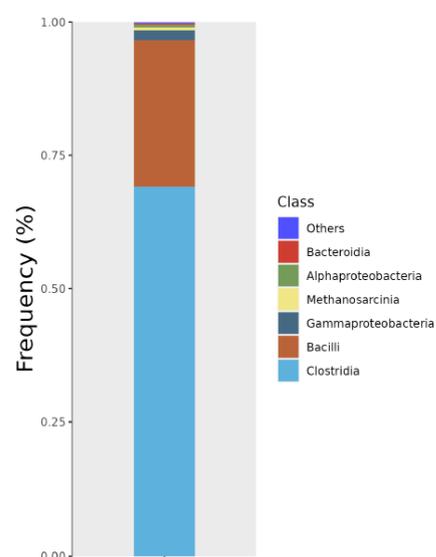
4.5.1 Taxonomy composition analysis

The most abundant phylum was Firmicutes and Proteobacteria 96.58% and 2.34% respectively. These are the most common phyla accounting for greater than 90% of abundance in the Anaerobic co-digestion process of OFMSW and bio-flocculated sludge is found. These

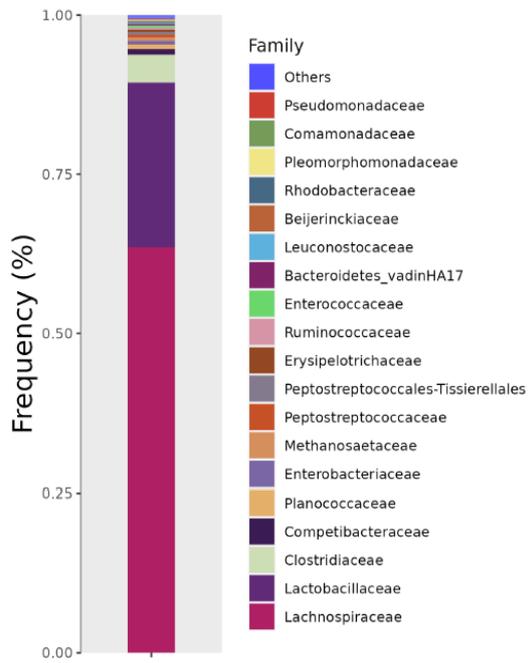
and proteins that compose lignocellulose make them candidates to transform low-cost, sustainable, lignocellulosic feedstocks (i.e., forestry, agricultural, and municipal wastes) into value-added biochemicals (Tom zaplana et al.,2024). Lachnospiraceae metabolize polysaccharides that can convert into ethanol and propanol and also release CO₂ and H₂ gases and generate butyrate, propionate, lactate, acetate, formate. Lachnospiraceae have been identified as biocatalysts for the conversion of biomass to hydrogen (Bu et al., 2021). In addition to its use as a fuel, hydrogen produced from lignocellulosic fermentation can be used as a reductant to fix CO and CO₂ by acetogens through gas fermentation. Lachnospiraceae and methanogens or acetogens have the potential for the direct conversion of lignocellulose to methane and other value-added biochemicals. At the genus level Anaerostignum (63.75%), Lactobacillus (25.74%) and Clostridium Sensu Stricto found with a high level of abundance level. Anaerostignum is of the same order level of Lachnospirales of the Lahnospiraceas family and from Firmicutes Phylum (Kulapong Jayanam et al., 2022) while Lactobacillus genus is from the Bacilli class. Clostridium sensu stricto is the main genus of Clostridium cluster I that belongs to the Firmicutes with the function of hydrolysis and acidogenesis which indicates that the hydrolysis and acidogenesis proceeded throughout Anaerobic Digestion (Xiaoshan Meng,2018)



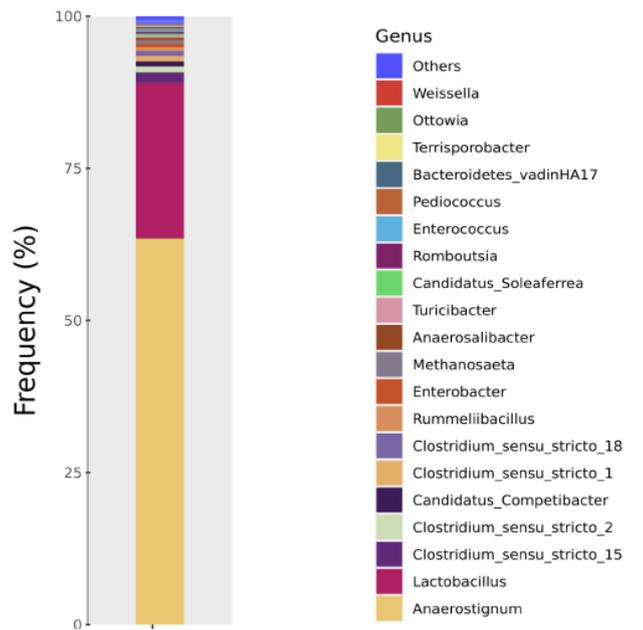
(A) Phylum



(B) Class



(C) Family



(D) Genus

Figure 33 : Abundance of bacterial community at (A) Phylum level (B) Class level (C) Family Level (D) Genus

CONCLUSION

Bio-flocculated sludge generated from SST (post-UASB) is the potential co-substrate for OFMSW for anaerobic co-digestion due to its characteristics of high pH 7.9 ± 0.2 , high moisture content $94.76 \pm 0.6\%$, low %TS 5.24 ± 1.18 , COD 52.22 ± 4.03 mg/gm and having potential to generate specific biogas yield 419.44 ± 11.59 L/kg VS_{added} under optimum condition. The anaerobic co-digestion process of OFMSW and bio-flocculated sludge operated with different mixing ratios shows high VS removal efficiency with a mixing ratio of 90:10 and 75:25 of 77.87% and 85.97% respectively. 75% wet wt. of OFMSW when co-digested with 25% of bio-flocculated sludge from SST can achieve a specific methane yield of 256.44 ± 12.98 L/kg VS_{added} and volumetric methane generation rate of 0.891 L/L/d which is quite higher than mono-digestion of OFMSW. The dynamic pattern of methane generation is approved by the presence of aceticalstic methanogens. Kinetic model Modified Gompertz model validate the experimental methane yield with non-linear regression with goodness to fit R^2 0.999 with prediction of maximum methane production rate ((L/kg VS_{added}*d) 137.49 and hydrolysis rate constant (k) 0.664. This shows acceptance of mixing ratio of 75:25 for anaerobic co-digestion of OFMSW and bio-flocculated sludge which is extremely satisfactory due to availability of substrate on field condition.

The lab-scale semi-continuous flow anaerobic reactor operated with a 75:25 mixing ratio of OFMSW and bio-flocculated sludge with varied %TS range and varied loading rate, reactor is performed well with an OLR range of 2 to 3 gm VS/L/d due to high biogas yield and %VS removal efficiency. Kinetic parameters are developed for cumulative biogas yield with First Order kinetic model, Modified Gompertz model and Logistic Function model with R^2 0.98 validate the experimental biogas yield for anaerobic co-digestion process for semi-continuous flow reactor. Kinetic constants obtained for semi-continuous flow anaerobic reactor with Stover Kin-cannon model (maximum removal rate constant $U_{max} = 31.74$, Saturation rate constant $K_B = 86.02$), Grau's Second-Order kinetic constants ($a = 0.578$, $b = 3.1$) contribute for prediction of substrate removal efficiency.

Prediction model developed using Feed Forward Neural Network (FFNN) fitting application (fitnet) simulate experimental data of %VS_{removal} and methane yield with goodness to fit R² more than 0.96 for anaerobic co-digestion process operated under semi-continuous flow while prediction model using ANN-PSO with R² of 0.80 and ANFIS with gauss2 membership function remarkable R² of 0.90 shows versatility of different modelling approach in accurately prediction of methane yield. FFNN modelling with finet application is quite an acceptable modelling technique due to its robustness and accuracy in prediction modelling techniques while using ANFIS requires very little time in the prediction of methane yield.

Metagenomic sequencing analysis can show the presence of Lachnospiraceae, Lactobacillaceae and Clostridiaceae are the dominant families with Anaerostignum, Lactobacillus and Clostridium Sensu Stricto genus found with high levels of abundance. They are dominant for the fermentation process of the anaerobic co-digestion process. Lachnospiraceae and methanogens or acetogens have the potential for the direct conversion of lignocellulose to methane and other value-added biochemicals.

Anaerobic co-digestion of OFMSW and bio-flocculated sludge from SST is a key to solving solid-liquid waste management problems with in-house substrate availability within the ULBs with minimal pretreatment to achieve waste-to-energy goals.

RECOMMENDATION

- Pilot-scale anaerobic digester using OFMSW and bio-flocculated sludge should be operated.
- Field-operated data can improve the accuracy of an Artificial Neural Network-based prediction model for methane yield.
- Life Cycle Assessment to evaluate environmental impact assessment and circular economy of the anaerobic co-digestion process of OFMSW and bio-flocculated sludge.
- Microbial insight using metagenomic analysis of each phase of the anaerobic co-digestion process of OFMSW and bio-flocculated sludge (post-UASB)

PUBLICATIONS:

1. Shroff, K. C., & Shah, N. G. (2023). The Performance Evaluation and Process Optimization of Anaerobic Co-digestion of OFMSW with Bio-flocculated Sludge from Secondary Settling Tank: A Key to Integrated Solid–Liquid Waste Management. *Waste and Biomass Valorization*. <https://doi.org/10.1007/s12649-023-02176-7>
2. Shroff, K., & Shah, N. (2024). Prediction Modelling to Enhance Anaerobic Co-digestion Process of OFMSW and Bio-flocculated Sludge Using ANN. *Pollution*, 10(1), 481-494. doi: 10.22059/poll.2023.365129.2065

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1. Kinjal Shroff, Nirav Shah, entitled "**Development of prediction model for the performance of Figure using Artificial Neural Network**" presented at the fourth international conference on Advanced Engineering Optimization Through Intelligent Techniques (AEOTIT) held by Department of Mechanical Engineering, Sardar Vallabhbhai National Institute of Technology, Surat, India during 28-30 September 2023.
2. Kinjal Shroff, Nirav Shah, entitled "**Comparative study of kinetic modelling for anaerobic co-digestion process for substrate removal efficiency**" presented at the third international conference on New Frontiers in Chemical, Energy and Environmental Engineering (INCEE-2023) conducted by Department of Chemical Engineering, National Institute of Technology, Warangal, Telangana, India during 24-25 November 2023.