Chapter IV- RESULT AND DISCUSSION

The present chapter describes 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 are checked by operating a semi-continuous flow reactor with varied OLRs. The performance results are validated with different kinetic modelling. The prediction model is developed using lab-scale experimental data using Artificial Neural Network (ANN).

4.1 Anaerobic co-digestion of OFMSW and bio-flocculated sludge(post-UASB): 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 and releases greenhouse gases. Waste produced in large Indian cities has the potential to be utilised 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 complimentary qualities and enhance the process outcome result(Prabhu & Mutnuri, 2016) .

This study focuses on the anaerobic co-digestion of OFMSW and bio-flocculated sludge from the Secondary Settling Tank (SST) in batch reactors. The study takes into account several ratios (% wet mass) of OFMSW and bio-flocculated sludge (50:50, 75:25, 90:10, 0:100, and 100:0), to check the performance of the reactor. In the current study, the sludge recovered from UASB after sewage treatment is utilised (100ml) as an inoculant to initiate rapid startup.

4.1.1 Effect on %Total Solids

The Total Solid (TS) content is an essential parameter to get the most biogas possible from the substrate. According to the presence of TS in anaerobic digesters, the digesters are considered

wet digesters (%TS<10), semi-dry anaerobic digesters (%TS=10 to15%) and dry anaerobic digesters (%TS >15)(Liotta et al., 2014). Liquid anaerobic digestion (%TS 5-10) yields more biogas than high solid anaerobic digestion (%TS > 15). A TS concentration of < 12% yields the maximum amount of methane produced by the substrate (Borowski, 2015). Anaerobic digestion of rapeseed oil cake shows a higher biogas yield of 3983ml at 20% TS concentration and beyond that biogas yield is decreased (Deepanraj et al., 2017b). The literature study investigates the effect of TS on volumetric biogas yield from sludge and reported that biogas yield increased with an increase in initial TS from 2% to 8%. However, biogas yield declines with 10% TS (An et al.,2017). Higher TS concentrations in substrate take 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 and 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 the 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 mass. When OFMSW is combined with 50% (of wet mass) bio-flocculated sludge from SST, the TS content is less than 10%. Methanogens require less time to acclimatise 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 TS concentration during the experiment, as seen in Figure 11, could be the addition of $NaHCO_3$ alkali to raise the pH during the process.
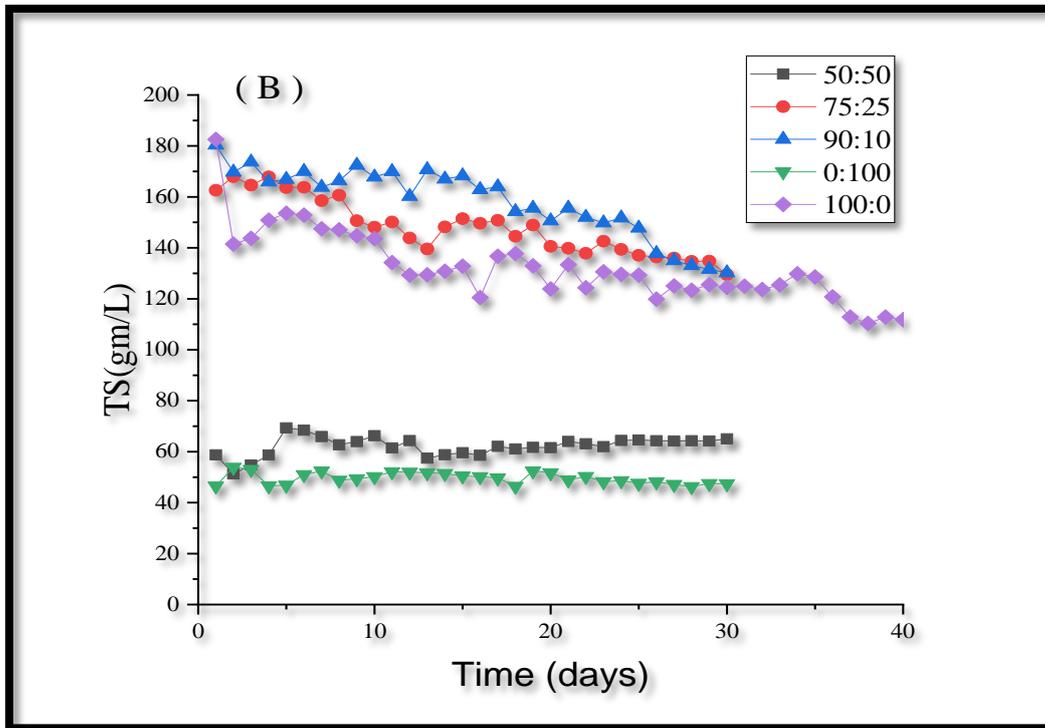
Figure 1: Effect on Total Solids (gm/L) during anaerobic co-digestion process

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 (VFA). 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 regularly and constantly to keep the pH in the range of 6.4 to 7.8. The optimum pH range in anaerobic digestion can be 6.9-7.3(Tchobanoglous et al., 2003) and 6.4-7.6 (Anderson & Yang, n.d.) Initial stage of the anaerobic digestion process pH drops in the reactor. The time taken for the anaerobic digestion process to neutralize is the adaptation time for bacteria(Syaichurrozi et al., 2013). If the pH value is lower or upper than the optimum range, it affects biogas production and reduces the methane concentration in biogas.

It has been noted during the investigation that co-digesting bio-flocculated sludge from SST with OFMSW helps to shorten the time for activation of the methanogenesis bacteria. The co-digestion mixing ratio of 50:50 (OFMSW: bio-flocculated sludge) requires less time to stabilise the pH of the anaerobic reactor than does the anaerobic mono-digestion of OFMSW. The bio-flocculated sludge from SST (after UASB) is the type of bio-flocculated sludge that may control pH in a few days without the need for alkali addition (Figure 12). 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 stabilise reactor operations for the methanogenesis phase.
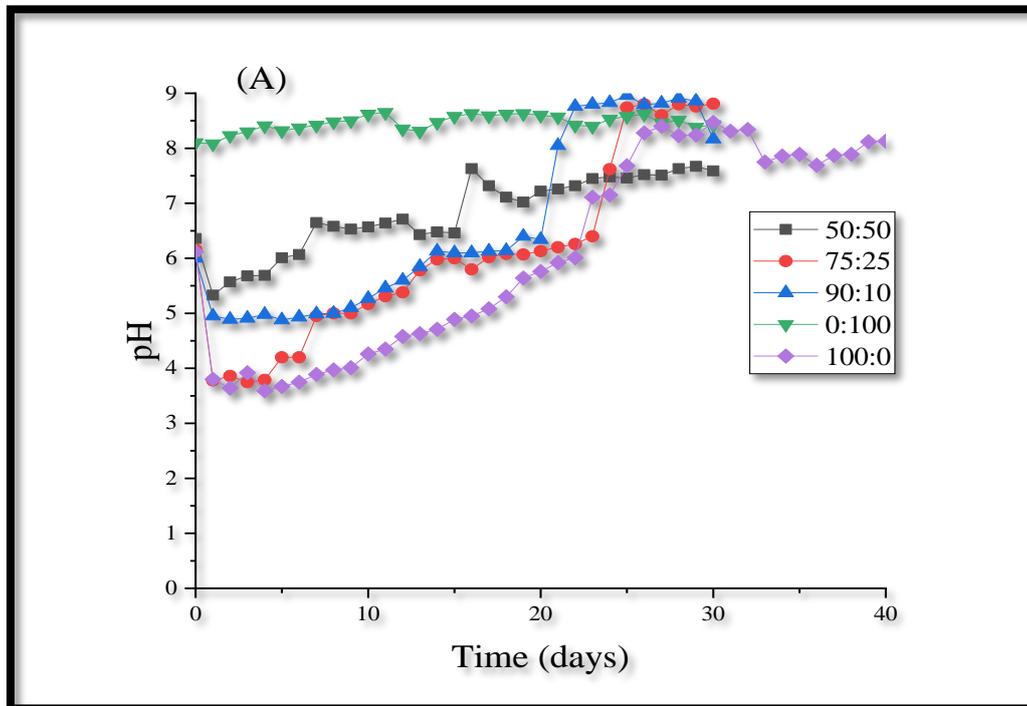

Figure 2: Effect on pH during anaerobic co-digestion process

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 considered, meaning that only volatile solids from input and outflow are considered. VS 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 a co-digestion mixing ratio of 90:10 and 75:25 of 77.87% and 85.97%, respectively. Anaerobic co-digestion with high TS concentration and mono digestion of OFMSW have a similar reduction in VS (Figure 13). When compared to mono-digestion of OFMSW at similar VS reduction, specific biogas yield is higher with a mixing ratio of 50:50,75:25,90:10. This can be because of higher production of VFA with more organic fraction in OFMSW, which inhibits the production of biogas. %VS$_{reduction}$ is calculated with considering the inorganic solids are not degraded during the anaerobic digestion process (Koch, 2015)

Primary sludge and fruit and vegetable waste when anaerobically co-digested under batch mesophilic conditions, VS removal efficiency is measured at 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., 2021). Dry anaerobic co-digestion of citrus waste, chicken feathers and wheat straw mixed with a ratio of 1:1:6 performs best with 238 ml/gmVS methane yield (Regina J. Patinvoh,2018).

4.1.4 COD removal efficiency

Chemical Oxygen Demand (COD) is the main metric used to evaluate the degree of environmental pollution. 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 SST and OFMSW is biodegradable on its own and may produce biogas but when co-digested, this can increase the output of biogas. The anaerobic co-digestion of OFMSW and bio-flocculated sludge from SST results in a COD removal efficiency enhancement of over $20.37\pm5.41\%$. Anaerobic co-digestion ratios of 50:50 and 75:25 show higher COD removal efficiency as compared to the mono-digestion of OFMSW. The total efficiency of an anaerobic batch reactor research is displayed for various mixing ratios in Figure 13.
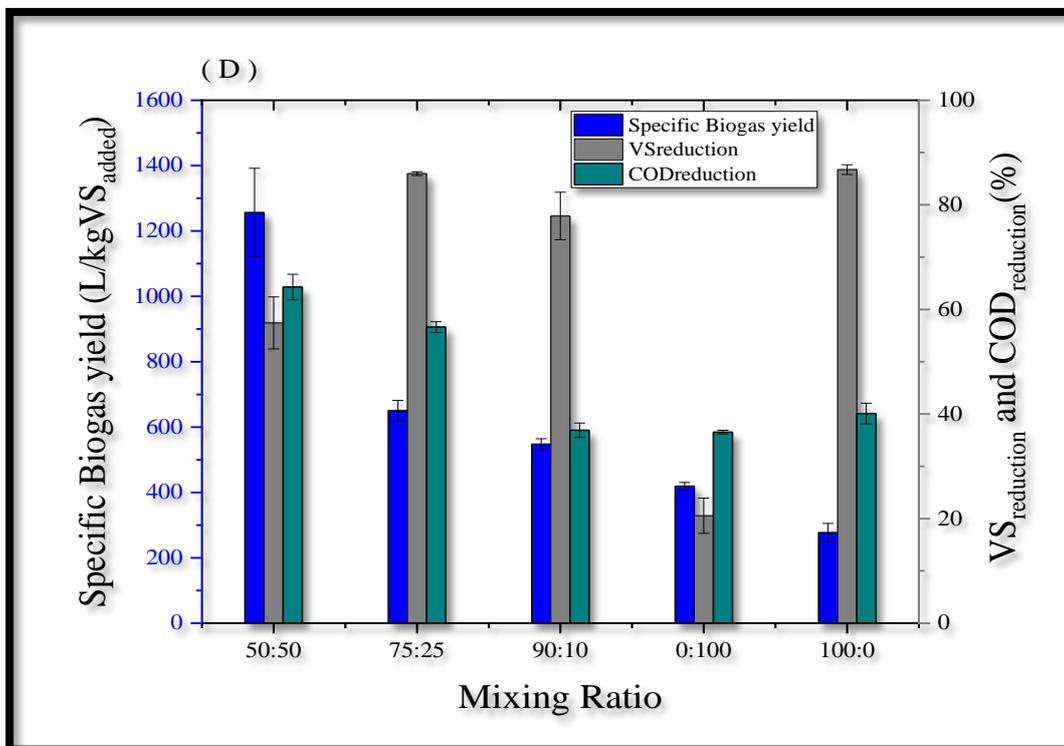
Figure 3:%VS reduction, %COD reduction, Specific Biogas yield (L/kgVSadded) for batch anaerobic co-digestion at different mixing ratios

4.1.5 Anaerobic reactor performance with ambient temperature condition

The temperature controllers in reactors are considered expensive and not preferred in developing countries. 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 between 25-35 °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 is 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 only for a few months of the year required a temperature controller. Here in this study, the temperature of an anaerobic reactor is controlled (30 °C to 35 °C) with a water jacketing system and heating rod.

4.1.6 Effect of mixing ratio on biogas yield for anaerobic co-digestion

Increasing the mixing amount of bio-flocculated sludge with OFMSW helps to raise the biogas yield. The yield of specific biogas production for the mixing 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 13 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 co-digestion of substrates. SBGY is determined by biogas generated (L) per VS supplied (gm/L) and VBPR (L/L/d) is the volume of biogas production (L/d) per volume of the substrate in the reactor (L) during the anaerobic co-digestion batch experiment.

 Anaerobic digestion produces two separate peaks for different mixing ratios of substrates (Dong et al., 2010). The first peak in the current study appears during the first five days of anaerobic co-digestion. It is followed by a second peak that appears on days seven, twenty-three, twenty-one, and twenty-four and steadily stabilises until the end of the experiment for different mixing ratios of 50:50,75:25,90:10 and 100:0. 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 (Figure 14). Previous research indicates that aceticlastic

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 aceticlastic methanogens, which provide a second peak during biogas generation. This demonstrates how the anaerobic co-digestion with varied mixing ratios causes a dynamic change in condition.
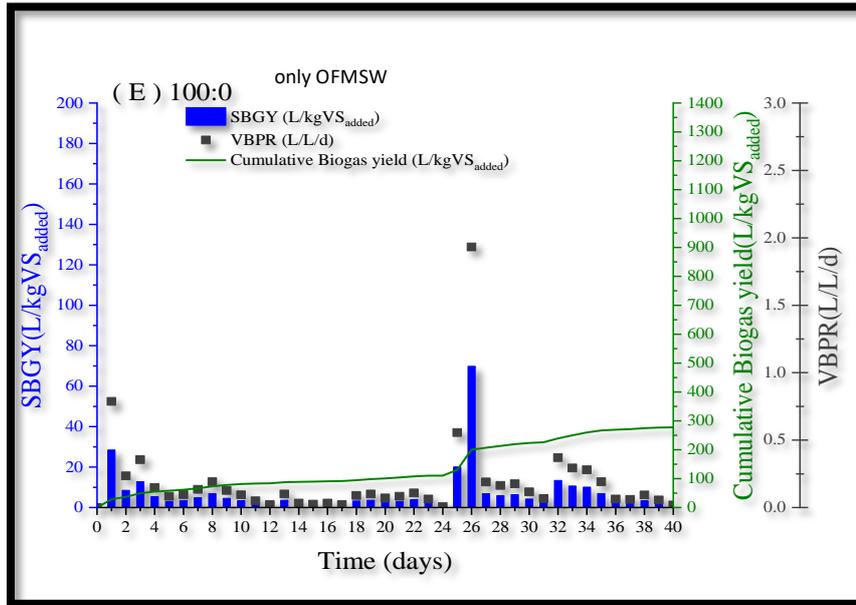
(C) 90:10

(D) 0:100

Only bio-flocculated sludge
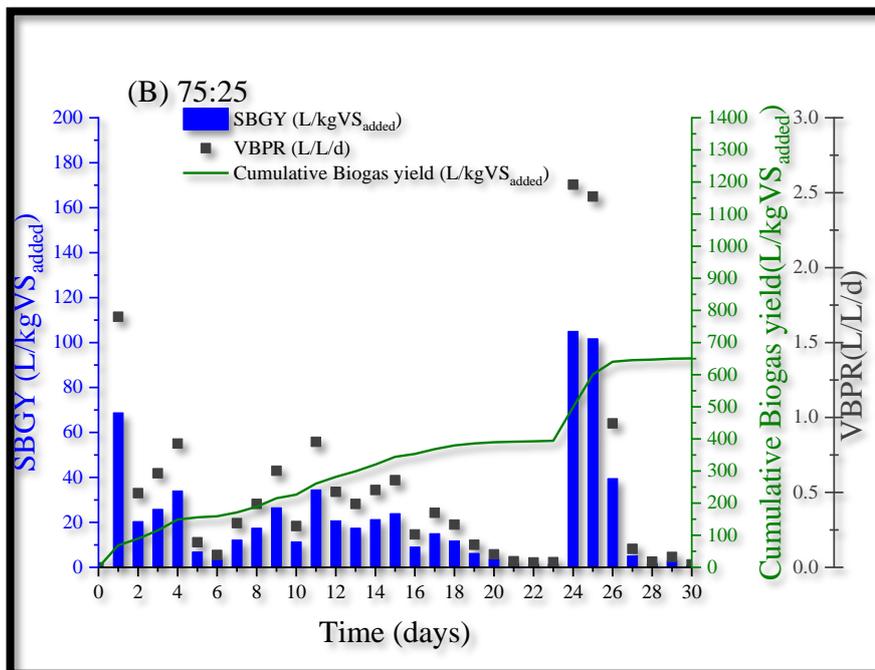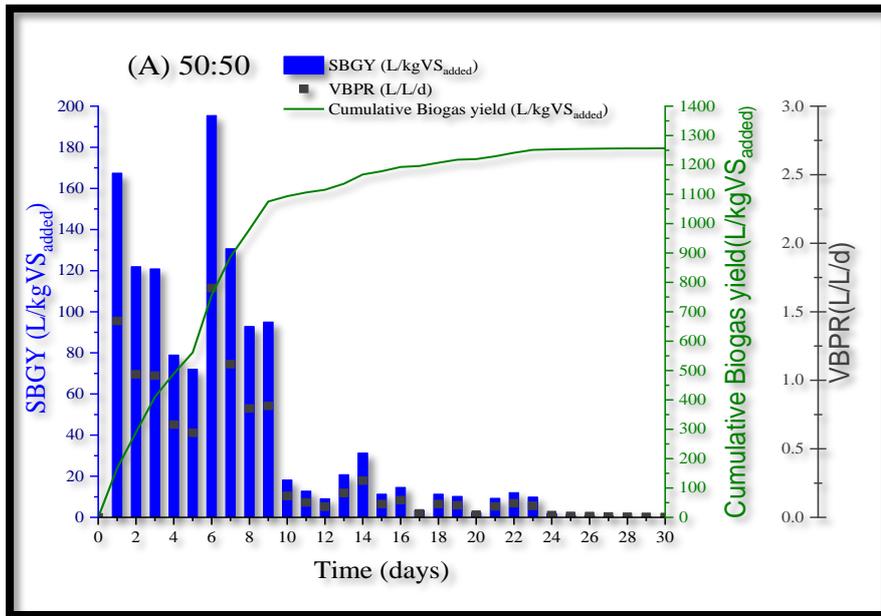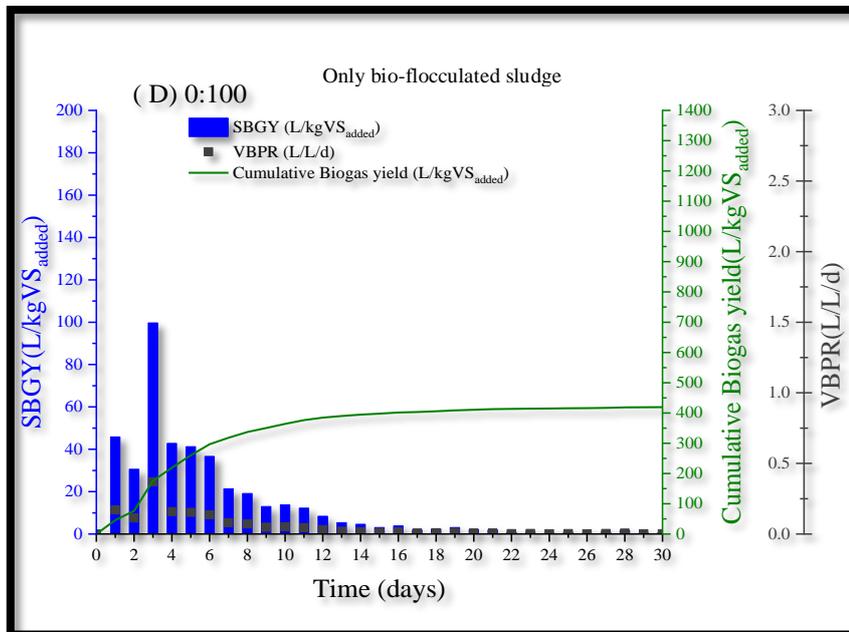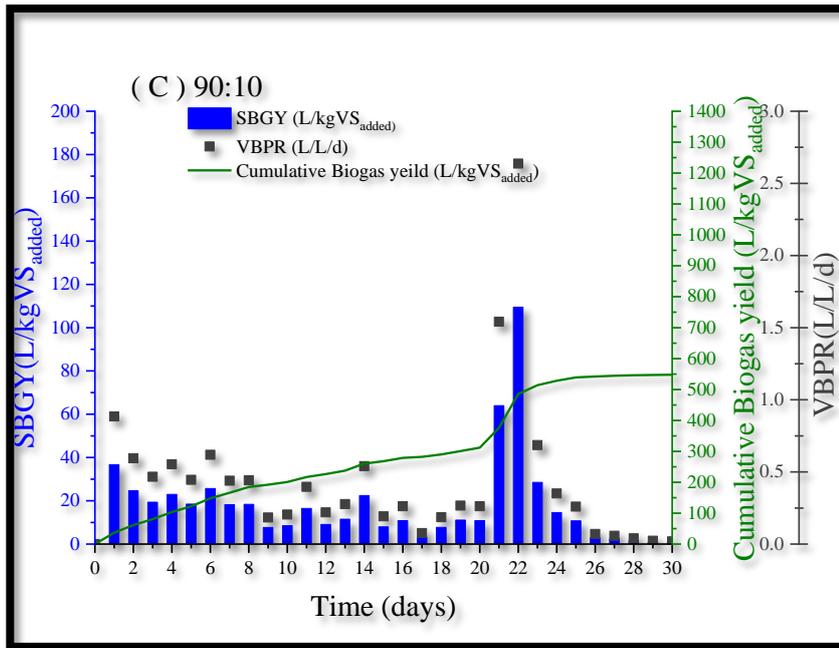
Figure 4: VBPR (Volumetric Biogas Production Rate) (L/L/d); SBGY (Specific Bio-Gas Yield) (L/kg VSadded); Cumulative Biogas Yield (L/kgVSadded) for different mixing ratios (A) 50:50 (B) 75:25 (C) 90:10 (D) 0:100 (only sludge) (E)100:0 (only OFMSW) of anaerobic co-digestion

When biogas is ignited, it flares up with a blue flame with pure methane gas produced at the second peak of the gas production process (Figure 15). 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 specific biogas yield.

Figure 5: Presence of methane gas during the methanogenesis phase

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 considered 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 anaerobic co-digestion study. 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 16.
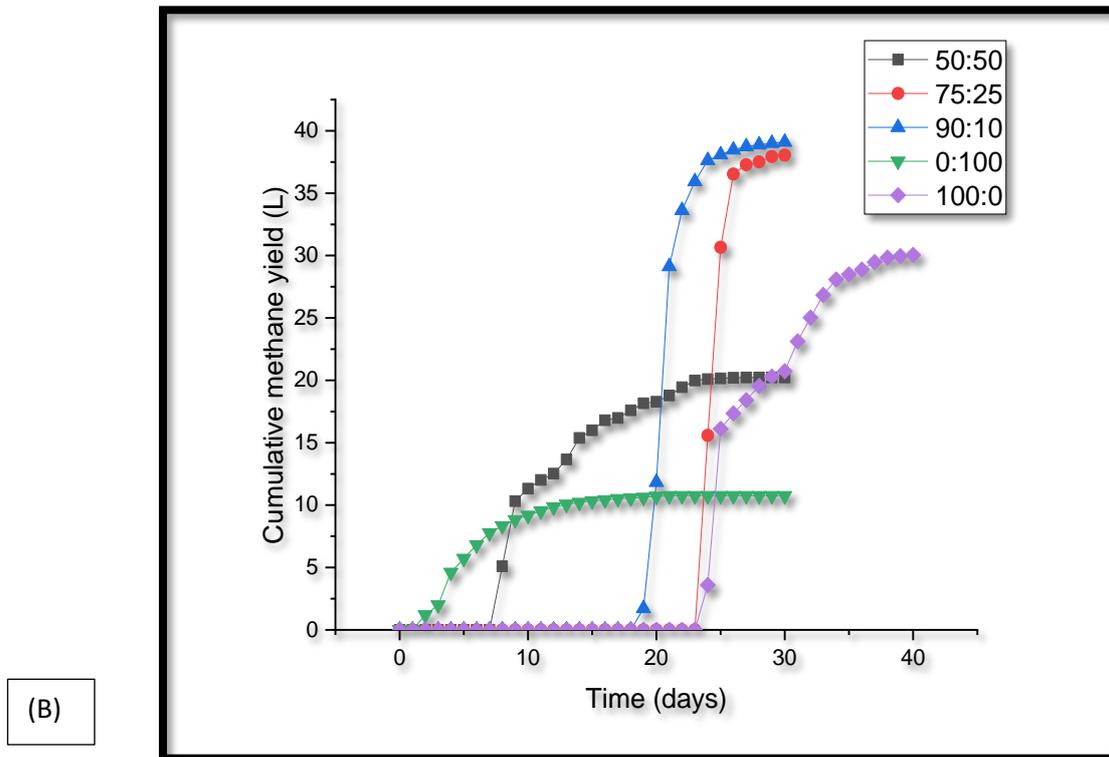

(A)

(B)
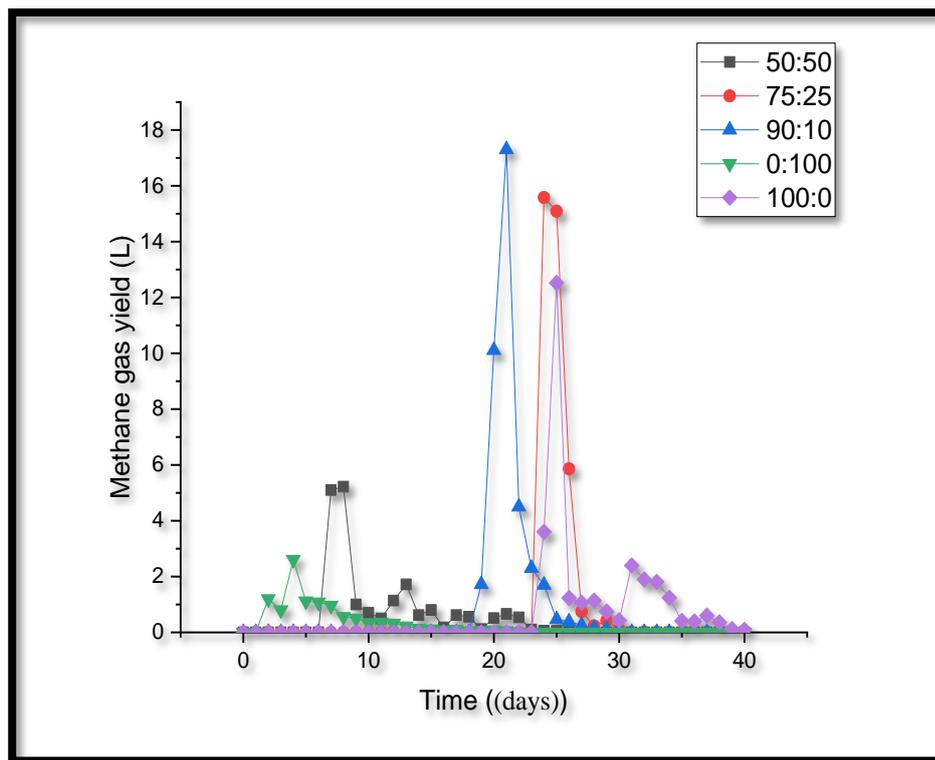
Figure 6:(A) Methane yield (L/d) (B) Cumulative methane yield (L) for batch anaerobic co-digestion with different mixing ratio

These outcomes are compared well to or surpass those of previous studies that used OFMSW co-digested with other co-substrates The bio-flocculated sludge from SST (post-UASB) has a specific methane yield of 373.41 L/kg VS_{added} which is comparable to 369 mL/gm VS methane yields of sewage sludge (Cabbai et al., 2013). When nightsoil and food waste are anaerobically co-digested in batch study with a mixing ratio of 70:30 generate 0.38 CH_4 gm COD /gm VSS day (Khanto & Banjerdkij, 2016), Brown water is combined with food waste in the same proportion (1:1) with the addition of inoculum sludge from WWTP achieving a biogas yield of 417 L/kgVS (Lim, 2011). OFMSW mixed with activated sludge and rice straw with the ratio of 1:1.5:1.5 in the batch study under the temperature of 37℃ generates 339.9 L/kgVS biogas. Food waste is co-digested with septic tank sludge (75:25) and cow dung is added as inoculum, resulting in a 471ml/gm VS biogas yield (Kesharwani & Bajpai, 2020). In batch anaerobic co-digestion under mesophilic conditions, food waste and sludge (before centrifuge) are co-digested and effluent from AD-treating food waste is used as inoculum, generating 823ml/gmVS biogas (Prabhu & Mutnuri, 2016).

4.1.7 Microscopic and Scanning Electron Microscopy imaging of methanogens

During anaerobic digestion, methanogens play a crucial role in methane gas generation. In earlier research, various methanogen species have been investigated during the anaerobic

procedure. The sampling is carried out during the methanogenesis phase of the reactor, to identify the presence of methanogens. Aceticlastic methanogens, which include species of both Methanosarcina and Methanosaeta, are typically present in the process. These methanogens use acetate as their primary energy source and produce methane as a byproduct. On the other hand, CO_2-reducing methanogens include species such as Methanobacterium, Methanothermobacter, Methanobrevibacter, Methanogenium, Methanocorpusculum, and Methanospirillum. These methanogens use CO_2 as their primary energy source and produce methane as a byproduct. In this investigation, microscopic images (Olympus-BX53) and SEM (Scanning Electron Microscopy) photographs of the methanogens are obtained, providing a detailed insight into their morphology.

A JEOL JSM-6380LV Scanning Electron Microscope is used to capture a distinct image of methanogens and their form. Figure 17 depicts that the methanogens are rod-shaped with flat ends. These forms suggest the likelihood of Methanosaeta's existence (Kim & Whitman, 2014) also known as Methanothrix. It is the most abundant methanogen and plays a significant role in methanogenesis. This order of microorganisms is considered the primary contributor to methane production through the acetoclastic pathway. It produces methane by converting not only acetic acid but also capable of using all methanogenic pathways to produce biogas (Arelli et al., 2021).
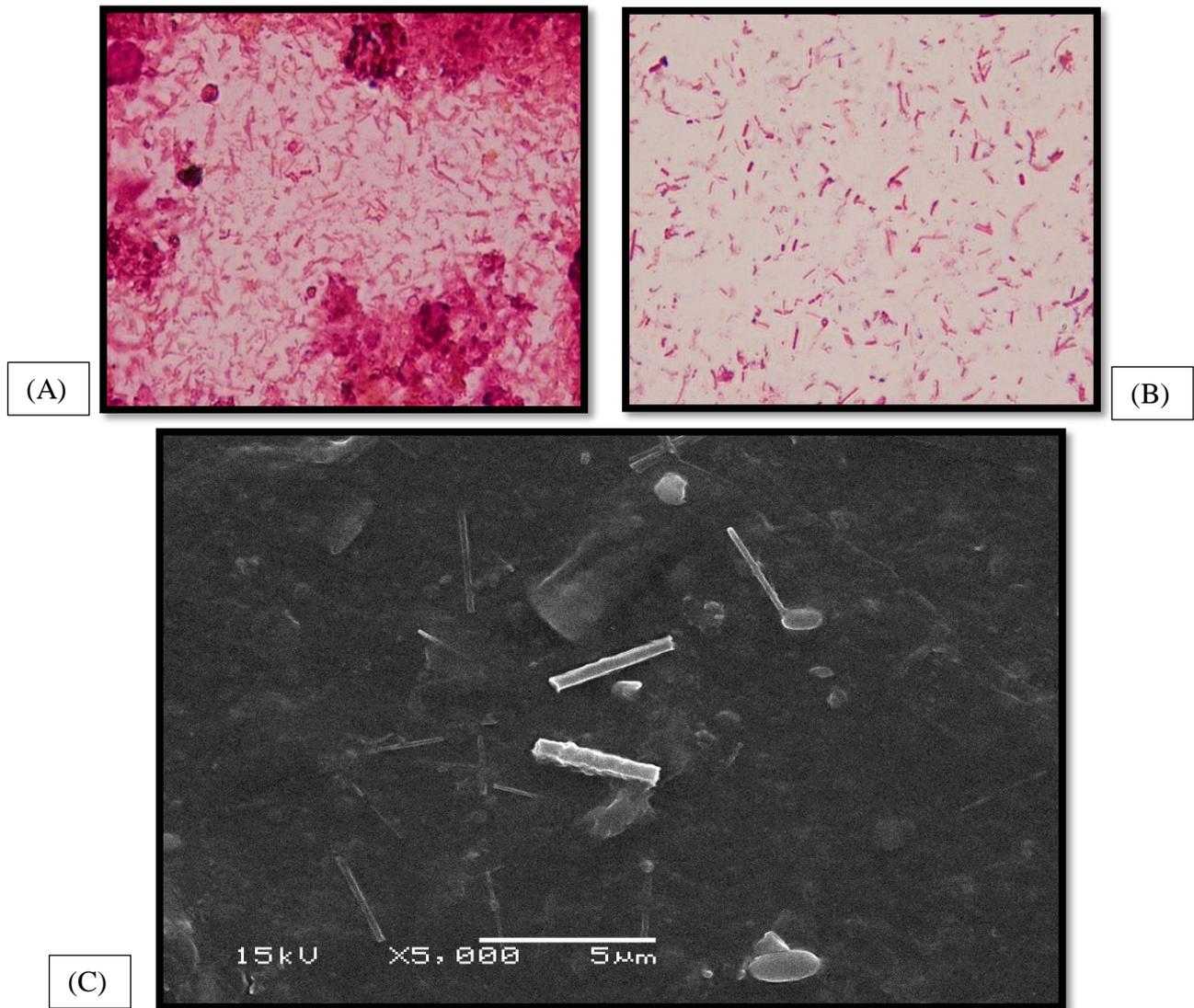
Figure 7: Microscopic analysis (A) Methanogens in whole sample (B) Methanogens in supernatant (C) SEM image of methanogens during the methanogenesis phase

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

The First-order kinetic is the simplest modelling technique used for complex substrates like sludge (Rao MS). It determines the rate-limiting step- hydrolysis for anaerobic digestion of complex material. The hydrolysis rate constant of organic materials can be determined using the First-Order kinetic model. Compared to mono digestion of OFMSW, the hydrolysis rate constant is higher in anaerobic co-digestion of OFMSW and bio-flocculated sludge at a mixing ratio of 75:25.

The two potential sources of the elevated hydrolysis rate constant are the increased biodegradability of the substrate and favourable environmental conditions for microorganisms for the entire degradation process. The maximum methane production rate (R_m) and lag phase (λ) are found by using Logistic Function modelling and the Modified Gompertz model, these models are useful in the evaluation of the kinetics of methane production (Ponsa S. et

al.,20011). The experimental and predicted data are fitted using the Modified Gompertz model with R^2 0.99 for various mixing ratios. The Modified Gompertz model shows no significant difference between experimental and predicted methane yield ($p<0.05$). Therefore, the experimental Methane yield is supported by this model.

The Modified Gompertz model and the Logistic Function model for different mixing ratios in the batch study of the anaerobic co-digestion process (Figure 18) results in methane yield from experimental data and predicted modelled data.
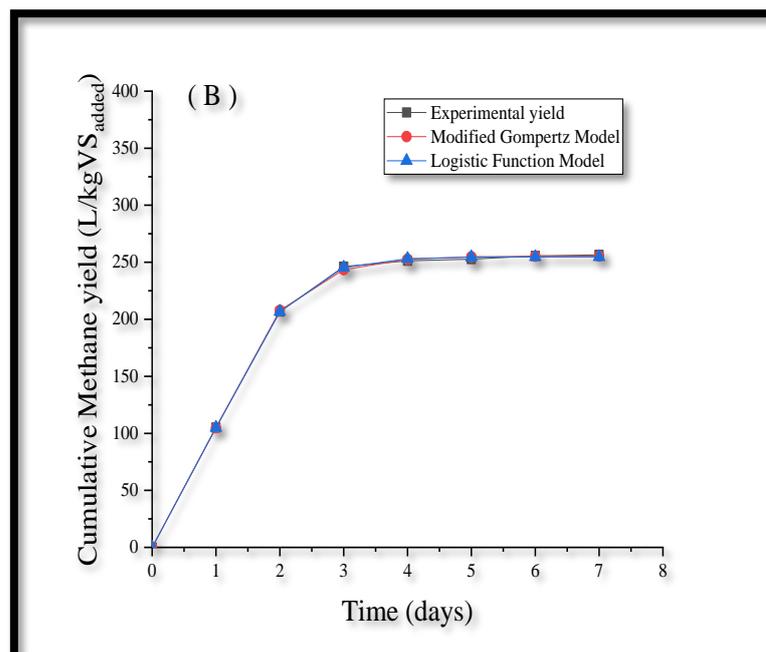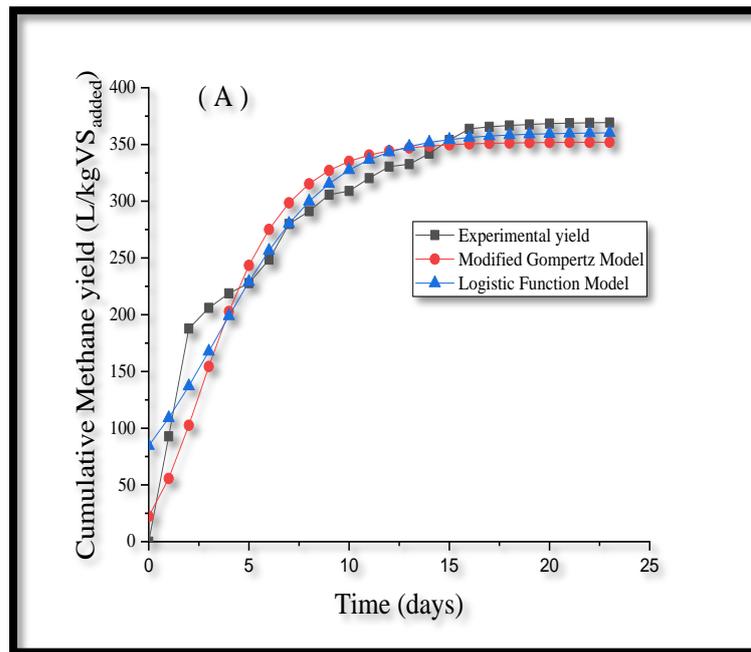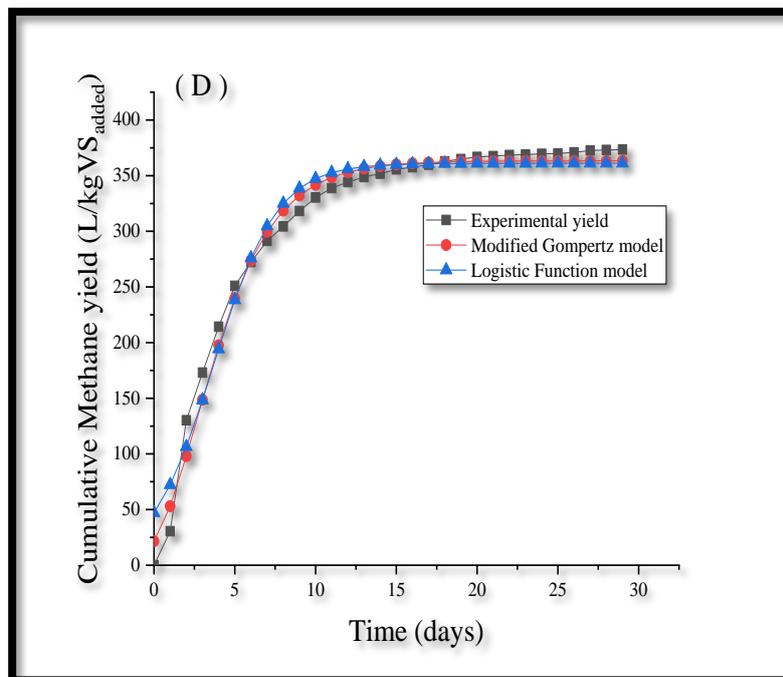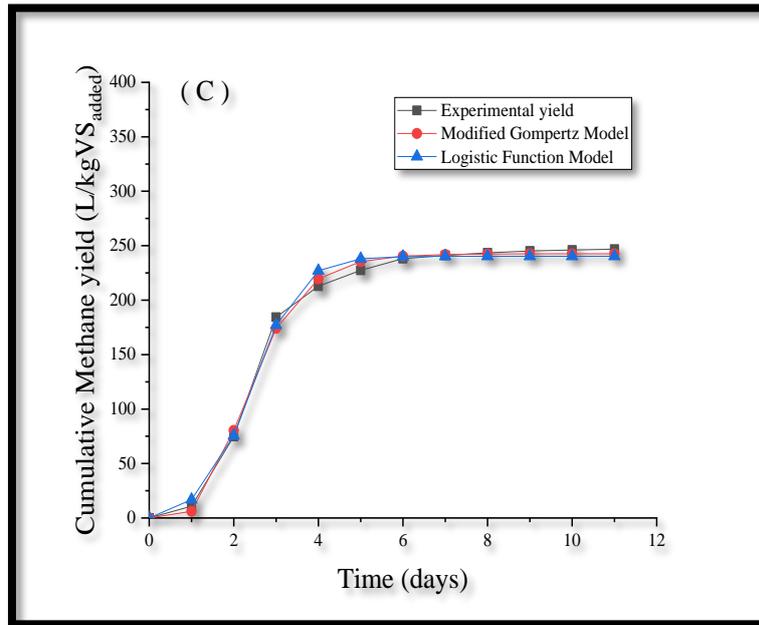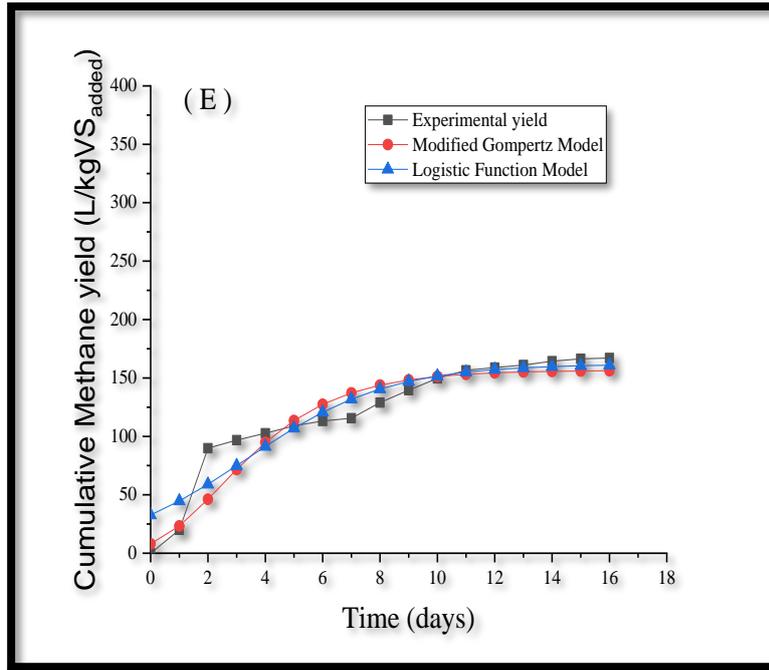
Figure 8: Plot of methane yield using kinetic modelling for different mixing ratios (A) 50:50, (B) 75:25, (C) 90:10, (D) 0:100, (E)100:0

Table 8 shows the kinetic parameters achieved using IBM SPSS Statistics 21 software where the hydrolysis rate constant (k) is higher with a mixing ratio of 75:25 with OFMSW and bio-flocculated sludge from SST (post-UASB) compared to other co-digestion mixing ratio and mono-digestion of OFMSW. Maximum methane production rate R_m (L/kg VS$_{added}$*d) can 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.

Table 1: Kinetic parameters with different mixing ratios of OFMSW & bio-flocculated sludge

	Mixing Ratio → Kinetic Parameters ↓	50:50	75:25	90:10	0:100	100:0
Hydrolysis rate constant	**k**	0.226	0.664	0.287	0.211	0.224
	R^2	0.9721	0.9833	0.9325	0.9948	0.9507
	P_m	352.06	255.39	242.58	363.28	156.58
	R_m	52.13	137.49	107.35	51.24	25.62

Modified Gompertz model	**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	**Pₘ**	1081.88	763.98	720.92	1083.76	485.51
	Rₘ	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

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

The anaerobic reactor has been operated with different OLRs for some 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. The variation in the composition of OFMSW affects the Total Solids (TS) of the substrate and subsequently affects the COD (mg/gm) and Volatile Solids.

4.2.1 Reactor start-up operation

Four stages hydrolysis, acidogenesis, acetogenesis and methanogenesis complete the anaerobic digestion process. An acclimatization period is required for the growth of microorganisms to reach the methanogenesis phase. 10L of an acrylic reactor is filled with 7.5kg OFMSW and bio-flocculated sludge from SST(post-UASB) with a mixing ratio of 75:25 (% wet mass). The reactor is operated for more than 200 days and its performance is observed with a mixing ratio of 75:25 for OFMSW & bio-flocculated sludge which is optimized during batch study (Shroff & Shah,2023). 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 the daily addition of alkali. The day the reactor is started, high production of biogas is observed which contains a very small 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 is a chance 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 starts feeding with different OLRs. Later OLR is increased and decreased according to the performance of the anaerobic reactor measured. At the same time, the composition of

OFMSW is also varied which leads to changes in substrate characteristics. The configuration, alignment and morphological composition of the substrate affect the biogas yield (Hegde & Trabold, 2019). The reactor is supplied the feedstock in semi-continuous flow with 8-8-8 hrs or 12-12 hrs dailyThe reactor is provided with a paddle mixer used for intermediate mixing of the substrate. Sample analysis is carried out daily, so 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 CO_2 gas generated in biogas.

4.2.2 %VS removal efficiency in a semi-continuous flow anaerobic 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 mass) 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 19 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 5 and 6 gm VS/L/d average %VS removal efficiency is observed between 60 to 70%. 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 the 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, the reactor is performed with higher %VS removal efficiency and later increasing OLR of 4 gm VS/L/d leads to a decrease in VS removal efficiency and remains 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 a very short period later decreases the pH and increases the VFA/Alkalinity ratio creating inhibition due to the accumulation of acids affecting the biogas yield. At OLRs of 8 and 12 gm VS/L/d, despite achieving transient high VS removal, the reactor exhibited instability.
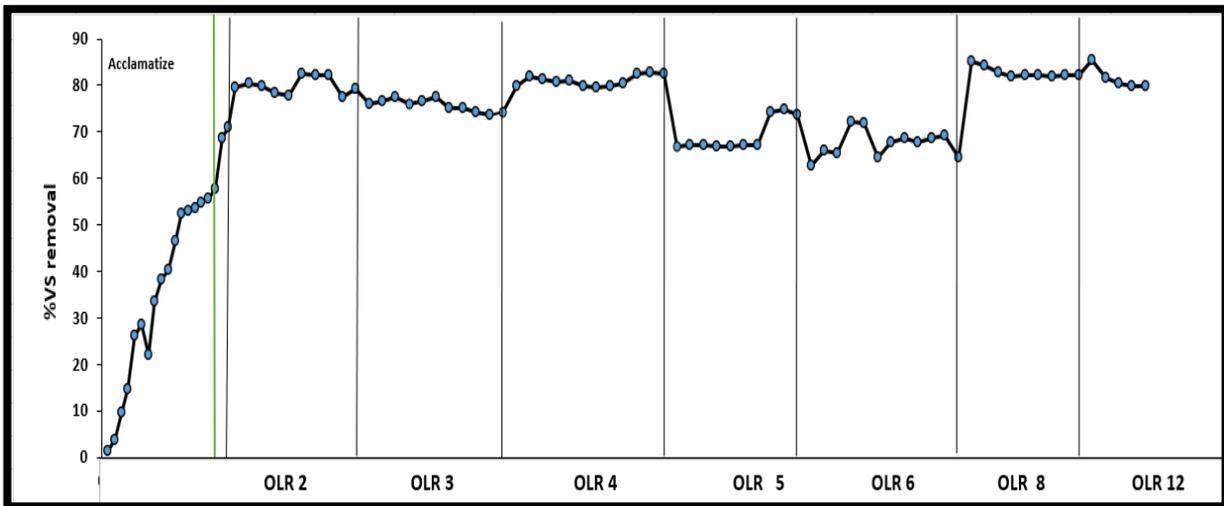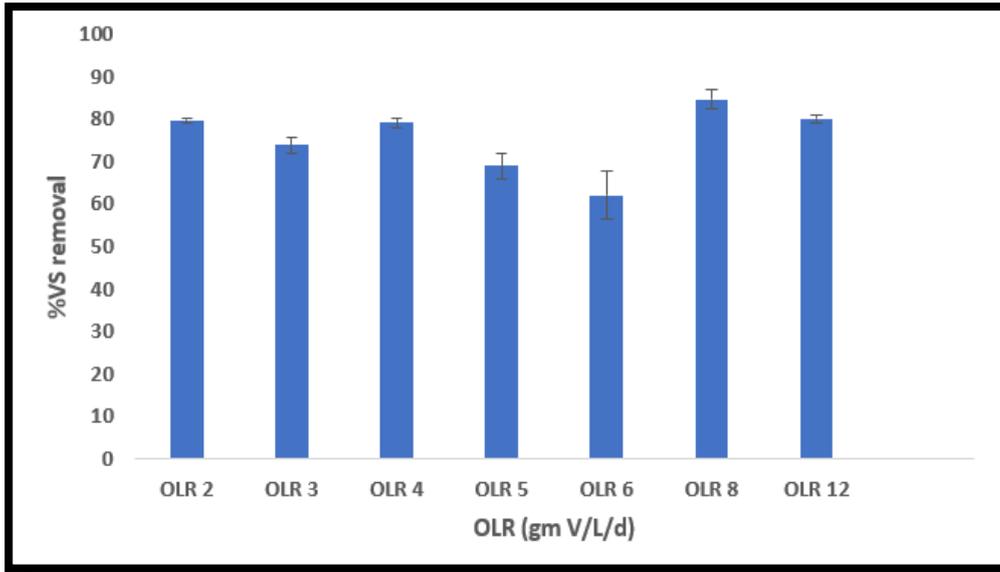
Figure 9: %VS removal efficiency at different OLR

4.2.3 Variation in pH

Figure 20 shows the average pH variation 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 emerged and a decrease in pH 6.5 and below.
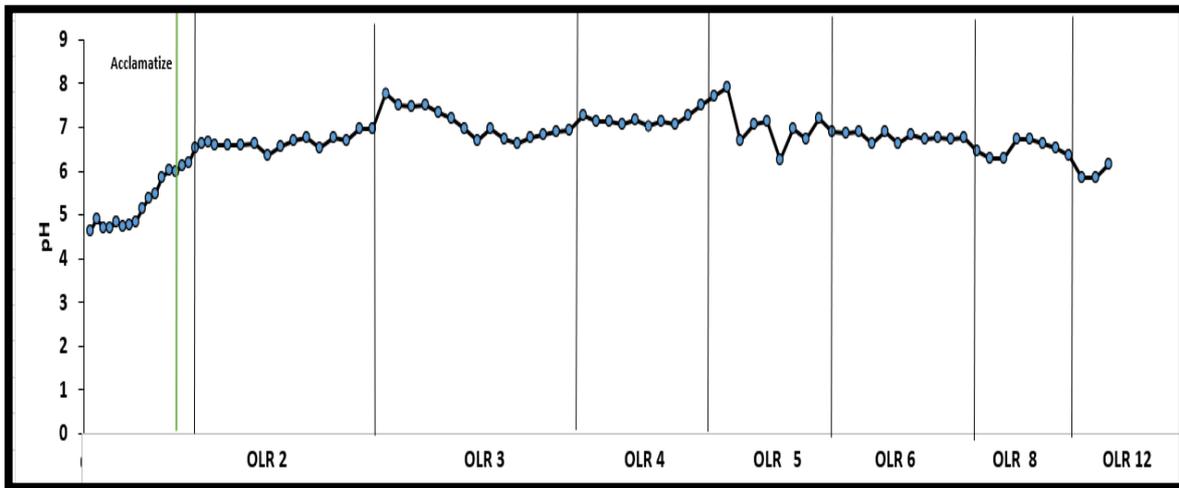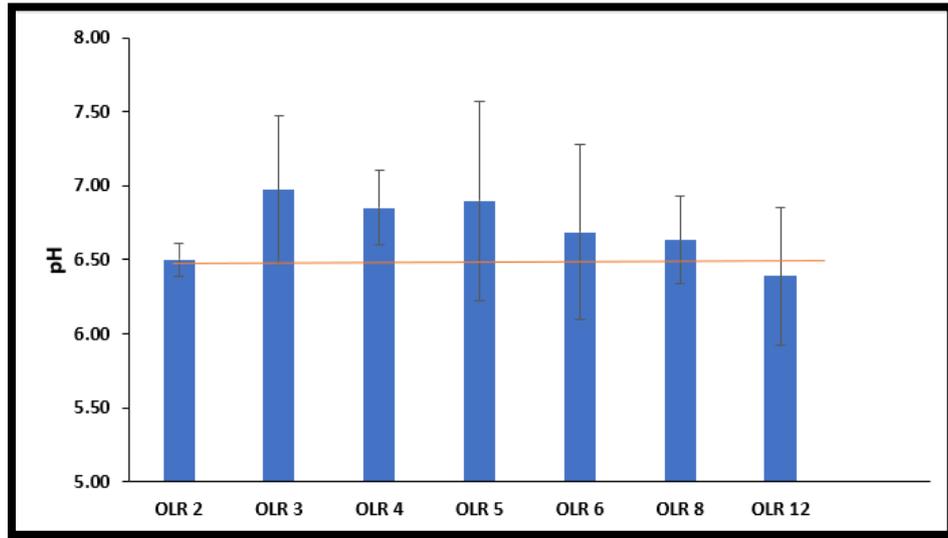
Figure 10: pH of the reactor for a semi-continuous flow reactor

For the critical activation of the methanogens in the anaerobic digestion process, pH must be 6.5 and above for optimal methane gas generation The observed reduction in pH with a higher OLR signifies the accumulation of VFA. This accumulation leads to a decline in pH and 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 for the performance of anaerobic co-digestion

VFA is an important indicator of the anaerobic reactor performance as it indicates a correlation between the VS breakdown and the presence of methanogenic bacteria. 85% of VFAs are mostly acetate, hence it becomes the most dominating VFA. Analysis of the VFA/Alkalinity ratio provides insight into the stability of the digester by maintaining a balance between the consumption rate and production rate. VFA concentration in an AD process particularly depends on the type of substrate introduced and the rate of organic loading applied during the operation of the anaerobic reactor. Excess production and accumulation of VFA in the anaerobic digestion process may reduce the pH and as a result, may inactivate the methanogens, hampering the performance of the reactor.
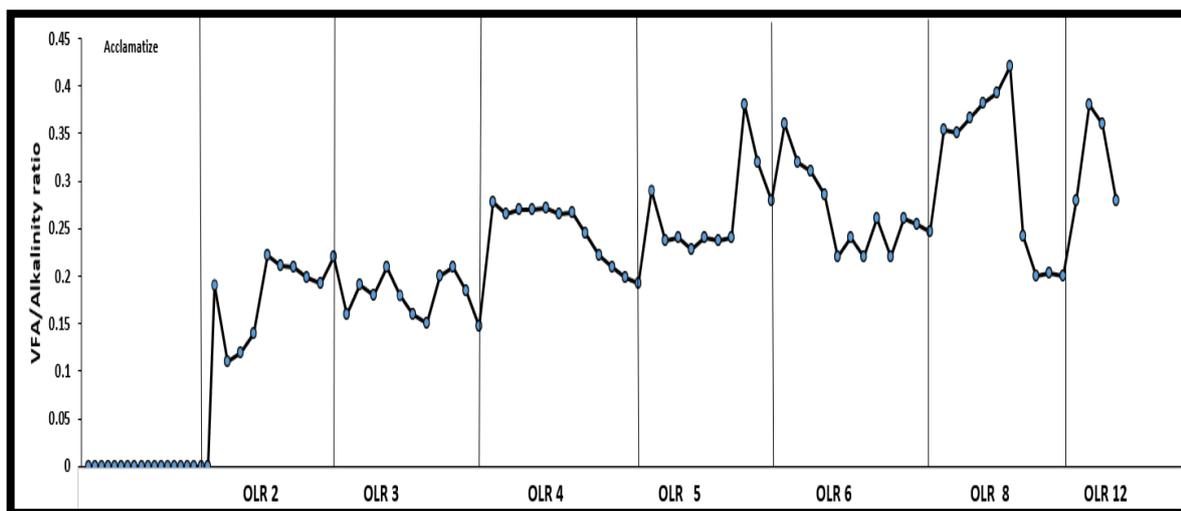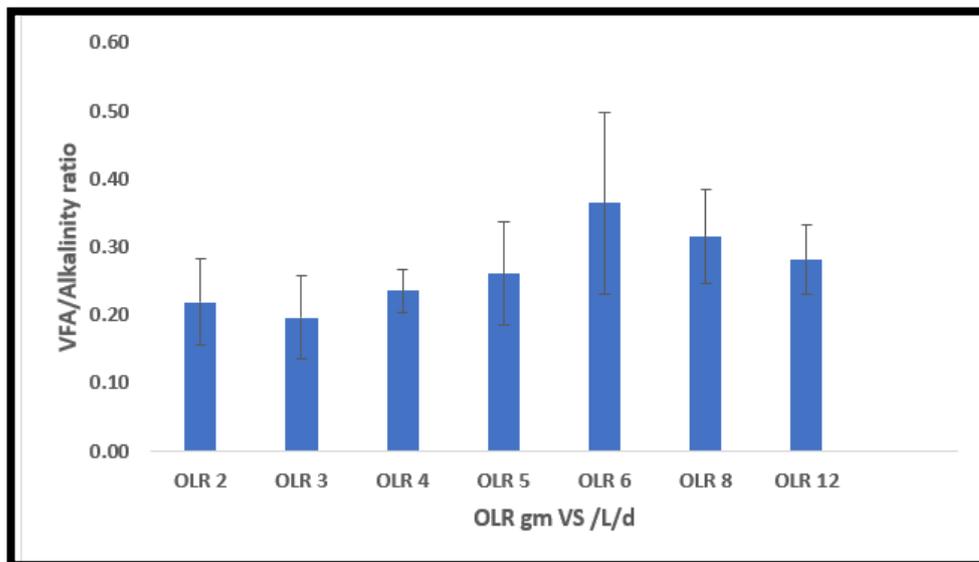
Figure 11: VFA/Alkalinity ratio for different OLR

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 to 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 (Figure 21). 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.

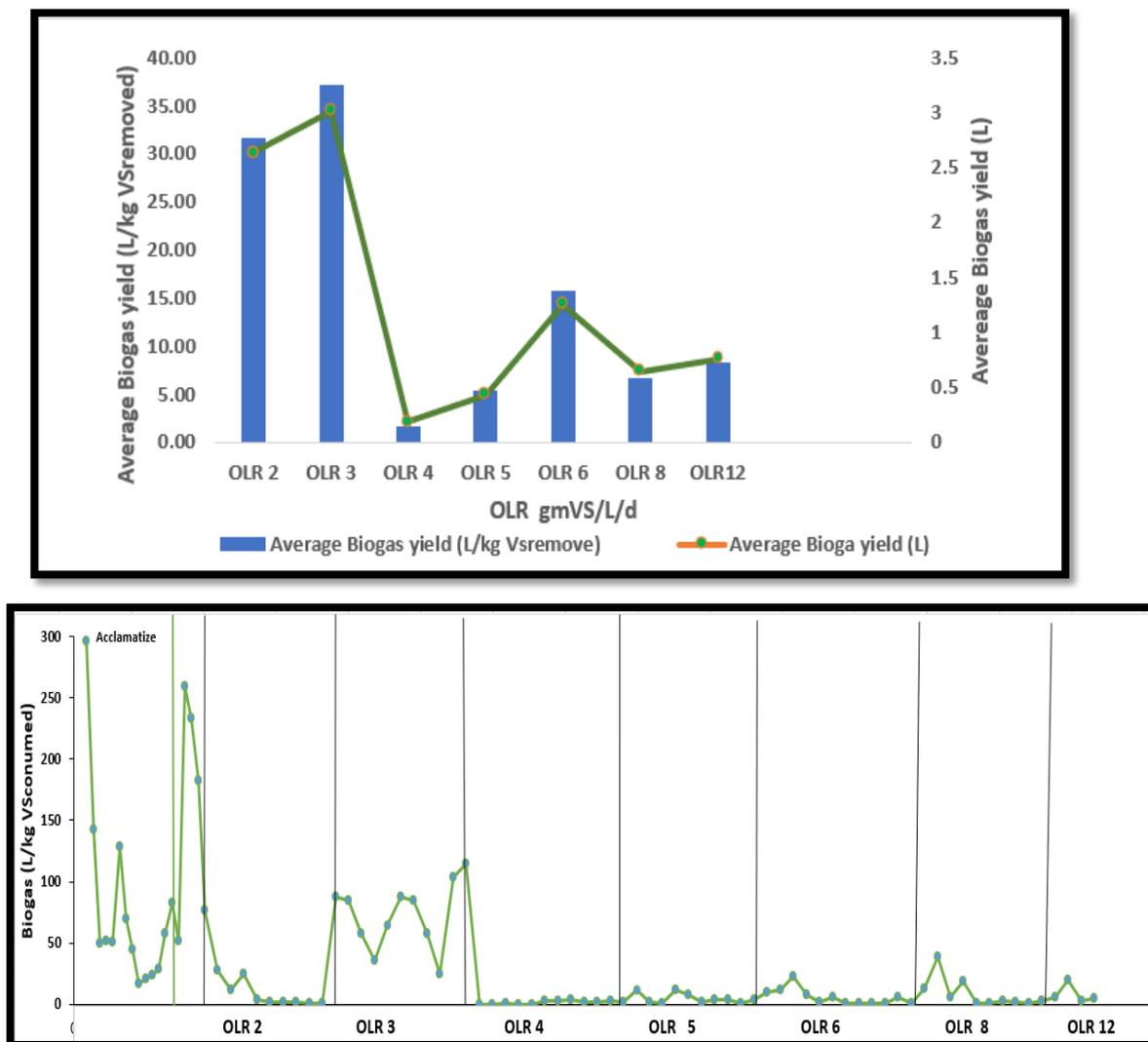**4.2.5 Biogas yield with different loading rates of the substrate**

Figure 12: Biogas yield for semi-continuous flow anaerobic reactor with varied OLR

Anaerobic co-digestion of OFMSW & bio-flocculated sludge with a 75:25 mixing ratio is used for the operation of a semi-continuous flow reactor. The observed biogas yield is an average of 31.68 and 37.20 L/gm $VS_{consumed}$ for OLR 2 & 3 gm VS/L/d respectively (Figure 22). This shows a positive trend with an increase in the loading rate increases the biogas yield.

However, if increase the OLR it reduces the production of biogas. The decline in biogas yields is due to the accumulation of VFA and a decrease in pH both of which can adversely affect the anaerobic co-digestion process. It is essential to note that sometimes when OLR is high, the biogas yield may be high for a very short period. However, this can lead to a sudden drop in the stability of reactor performance due to an increase in VFA/Alkalinity ratio and a decrease in pH which also halts the generation of biogas. To reboot the reactor operation, it is required to reduce the organic loading rate. This indicates that biogas generation varies at different OLRs in the anaerobic co-digestion process. The delicate balance required in OLR for constant biogas production while avoiding potential inhibitory effects of VFAs and pH.

The operation of an 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 changes in the characteristics and composition of the substrate which are 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 that affect the operation of the anaerobic reactor. %TS ranges from 12% to 23% and also once the substrate is fed with 32% Total Solids content as per availability of OFMSW in the field. Throughout the fluctuating %TS range and varying loading rate, the reactor performed 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 observed correlation between %TS variations, loading rates and reactor performance underscores the significance of substrate composition for achieving a stable and efficient anaerobic digestion process. 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 anaerobic reactor

4.3.1 Kinetic modelling for cumulative biogas yield

The present study uses three popular models namely the First-Order kinetic model, the Modified Gompertz model and the Logistic function model for predicting cumulative biogas yield during the anaerobic co-digestion process (Figure 23). A new hybrid kinetic model that combines the first-order kinetic model and logistic function is developed and applied to experimental data which can predict a more accurate cumulative biogas yield with R^2 0.99 compared to other kinetic models. Kinetic models validate the semi-continuous flow of anaerobic reactor 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 hybrid model as a valuable tool for enhancing the management of the anaerobic co-digestion process ultimately contributing to sustainable waste-to-energy solutions (Figure 24).

A modified hybrid model is developed to fit the experimental data. A modified hybrid kinetic model (Equation 12) is developed with a first-order kinetic model and a logistic function model. With the modified hybrid kinetic model, we can achieve higher hydrolysis rate constant k with goodness to fit (R^2) 0.99

$$M = P * \frac{(1 - \exp(-k*t))}{(1 + \exp\left(4*Rm*\frac{L-t}{Pm}\right) + 1}$$
 Equation 12

The equation combines the exponential decay of the First-Order model with the logistic function model. The numerator of the equation represents the cumulative biogas production that gradually increases over time and follows exponential growth. The denominator of the equation represents the logistic function model following a sigmoidal growth curve for biogas production. This equation provides a comprehensive representation of the kinetics of anaerobic digestion, including the initial exponential growth and the subsequent plateauing as the system approaches its maximum biogas production. Kinetic parameters k, P_m, L and R_m are determined using experimental data. The applicability of this kinetic model may vary depending on the operational conditions and characteristics of the substrate being modelled.
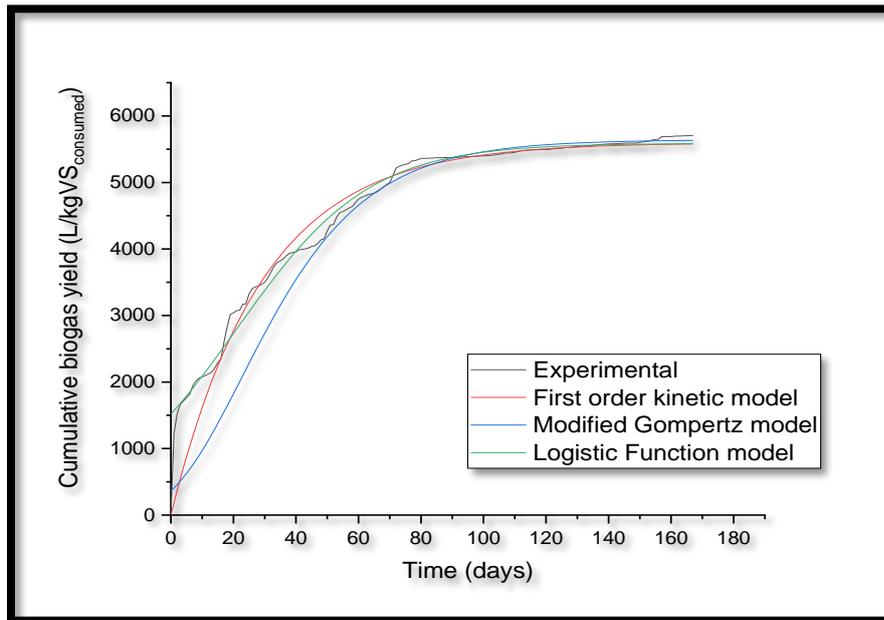
Figure 13:Kinetic modelling for cumulative biogas yield for semi-continuous flow anaerobic reactor
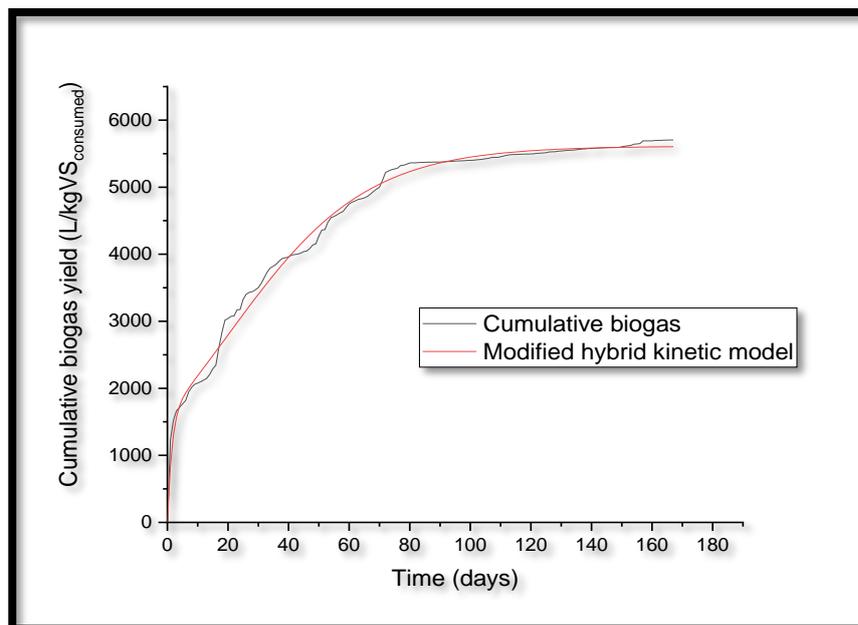

Figure 14: Modified hybrid kinetic model for cumulative biogas yield

Table 9 shows the kinetic parameters achieved with kinetic modelling for cumulative biogas yield of semi-continuous flow anaerobic reactor for lab scale experimental study. The kinetic constants developed for an anaerobic co-digestion semi-continuous flow reactor where both the model show higher accuracy while the new modified hybrid model shows also a higher hydrolysis rate constant.

Table 2: Kinetic constant for semi-continuous flow anaerobic digestion of OFMSW & bio-flocculated sludge

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
L	-	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 the 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 informed decisions about factors like the Organic loading rate (OLR) to achieve the desired COD removal rates and biogas production levels. Figure 25(A) shows the developed Modified Stover-Kincannon model. Here Equation 13 shows developed Modified Stover-Kincannon model achieves substrate removal rate constants $U_{max} = 31.74$ and $K_B = 86.02$ with R^2 0.86

$$Se = Si - \frac{31.74 * Si}{86.02 + (Q * \frac{Si}{V})} \qquad \text{Equation 13}$$
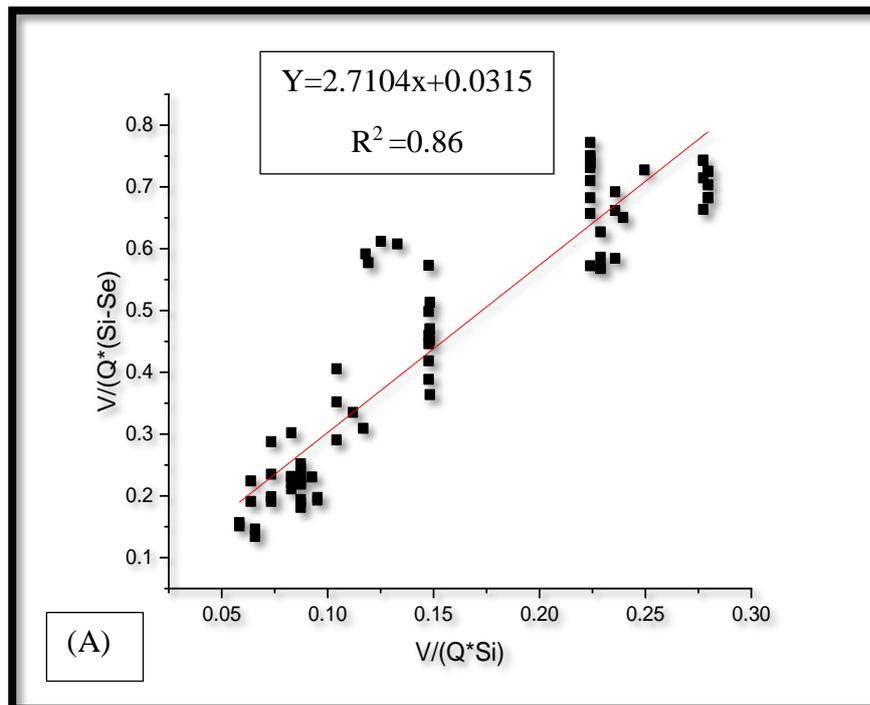
4.3.2.2 Grau's Second Order model

The plots of HRT vs HRT/COD$_{removal}$ to get Grau's Second Order kinetic constant a and b, are shown in Figure 25(B). A strong correlation coefficient ($R^2 = 0.90$) 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. The linear component of COD elimination is linked to this parameter, which may be tuned to improve process efficiency. Equation (14) predicts the COD content of the effluent substrate.

$$Se = Si * (1 - \frac{HRT}{0.578 + 3.1 * HRT})$$ Equation 14

4.3.2.3 First-Order kinetic model

The generated First-Order kinetic model is shown in Figure 25 (C). The First-Order rate constant k_r 0.12 is obtained with R^2 0.80. A higher kr value indicates a faster rate of COD removal which may be desirable for quicker waste degradation and biogas production. The correlation coefficient (0.80) indicates that the First-Order kinetic model cannot be applied precisely.

(B)

Y=3.1022+0.5783

$R^2 = 0.90$

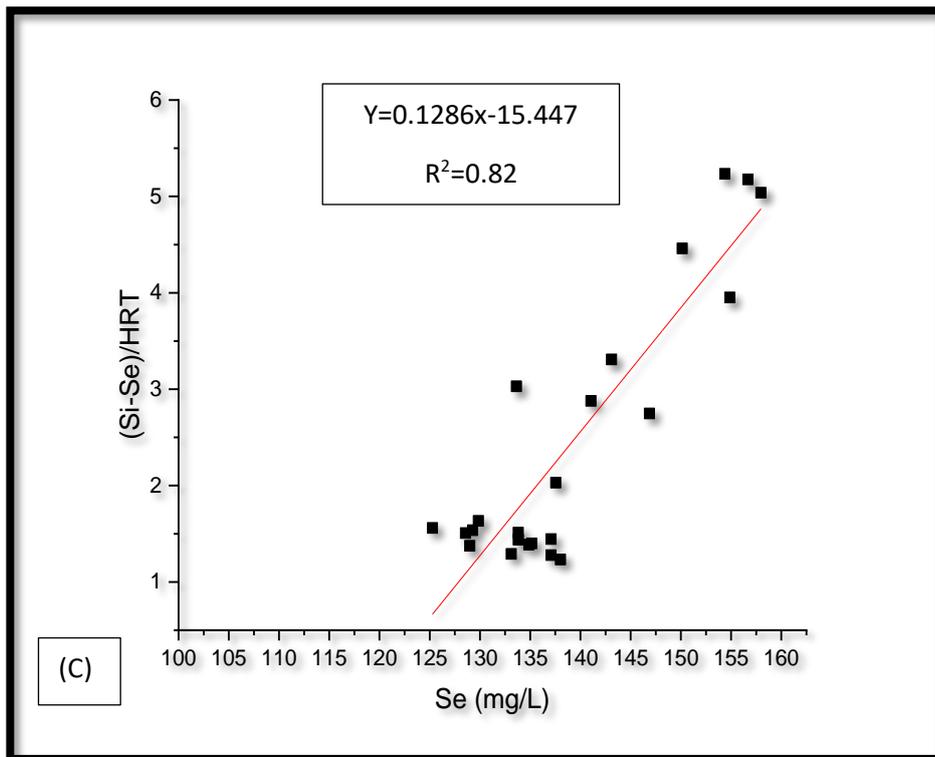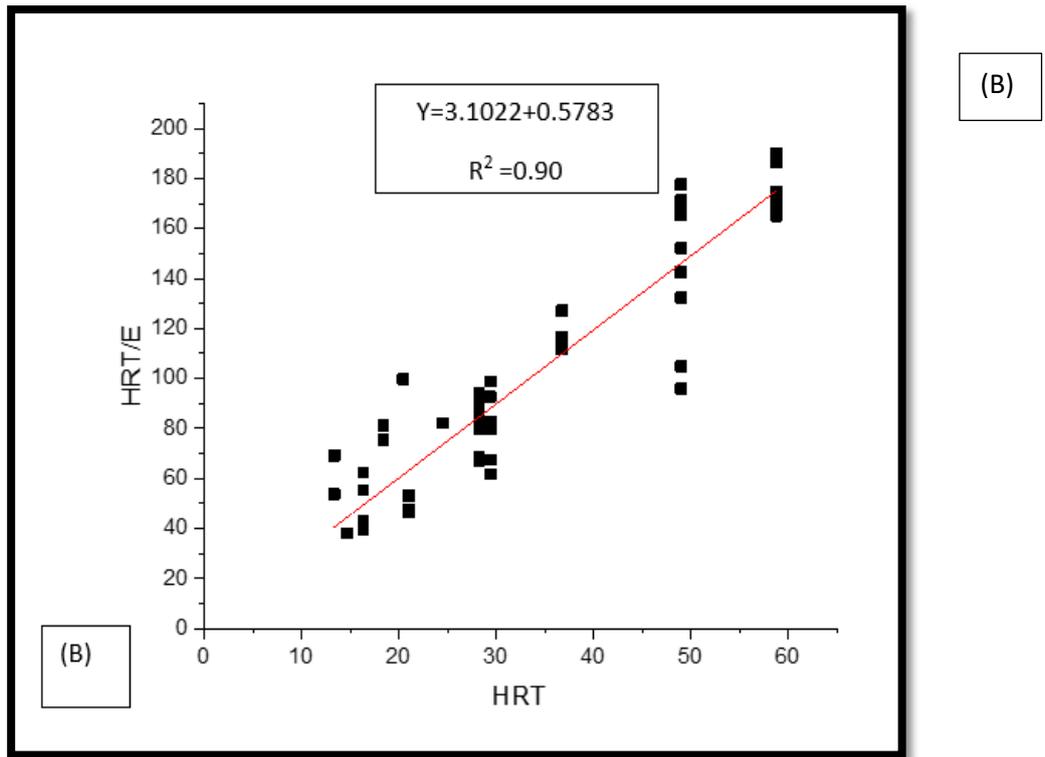(B)

Y=0.1286x-15.447

$R^2 = 0.82$

(C)

Figure 15: Plot for evaluation of kinetic model (A) The Modified Stover- Kincannon model
(B) Grau's Second-Order kinetic model (C) First-Order kinetic model

Table 10 shows the comparative study of kinetic constant for different substrates in earlier research.

Table 3: Comparative study of kinetic constants with different literatures

Substrate	Type of Reactor	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., 2019b

4.3.3 Kinetic modelling for prediction of effluent Volatile Solids with Modified Stover-Kincannon model and Grau's Second-order kinetic model

Kinetic model Modified Stover- Kincannon applied for prediction substrate removal on Volatile solids basis. Biogas generation is directly dependent on the degree of degradation of

volatile solids. Prediction of volatile solids in effluent is an important parameter to understand the anaerobic reactor performance. Modified Stover-Kincannon model applied for semi-continuous flow anaerobic digestion process to calculate kinetic constant for volatile solids in effluent. Volatile Solids are dependent upon the Total Solids of the reactor. The kinetic constant achieved with Volatile Solids Umax &K_B are 72.46 and 130.43 respectively with R^2 0.93
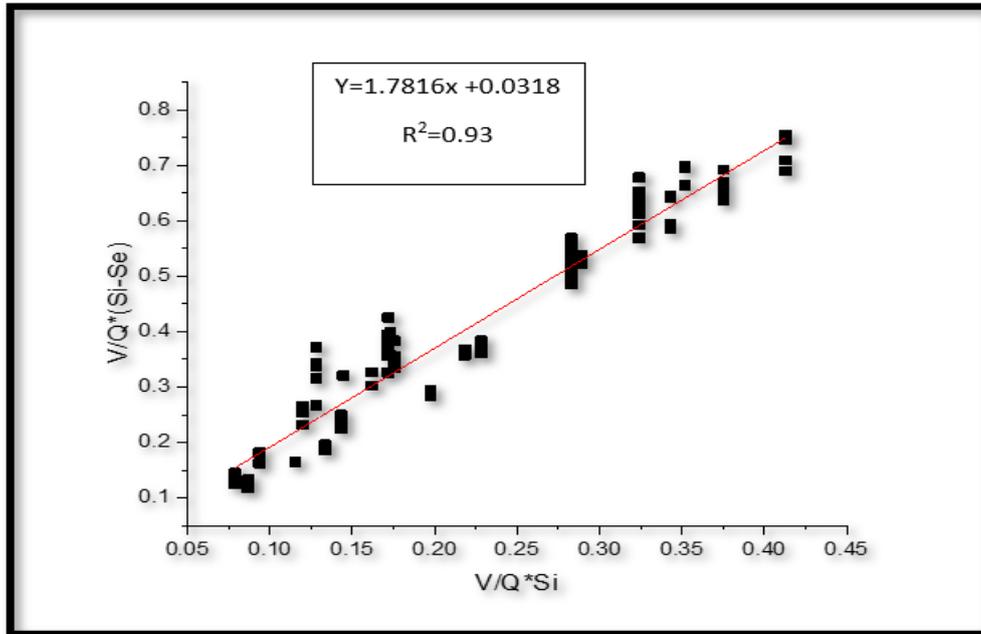

Figure 16: Modified Stover- Kincannon Model for Volatile Solids output
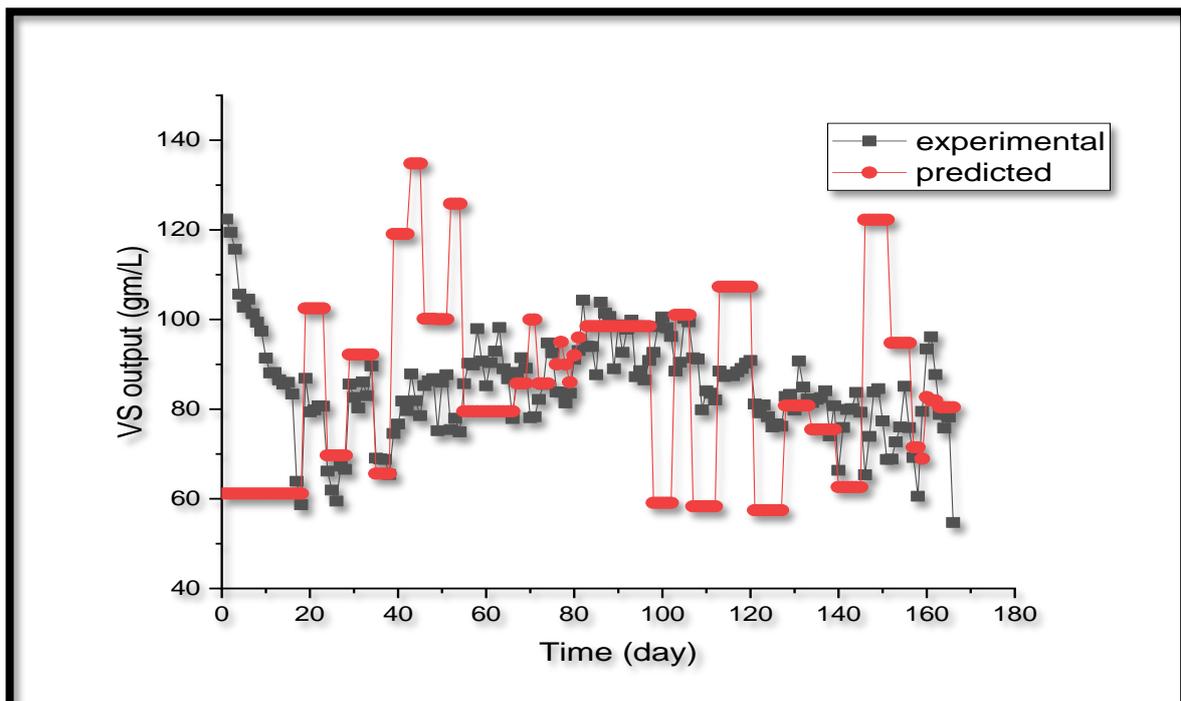
Figure 17: Experimental and Prediction Volatile solids output (gm/L)

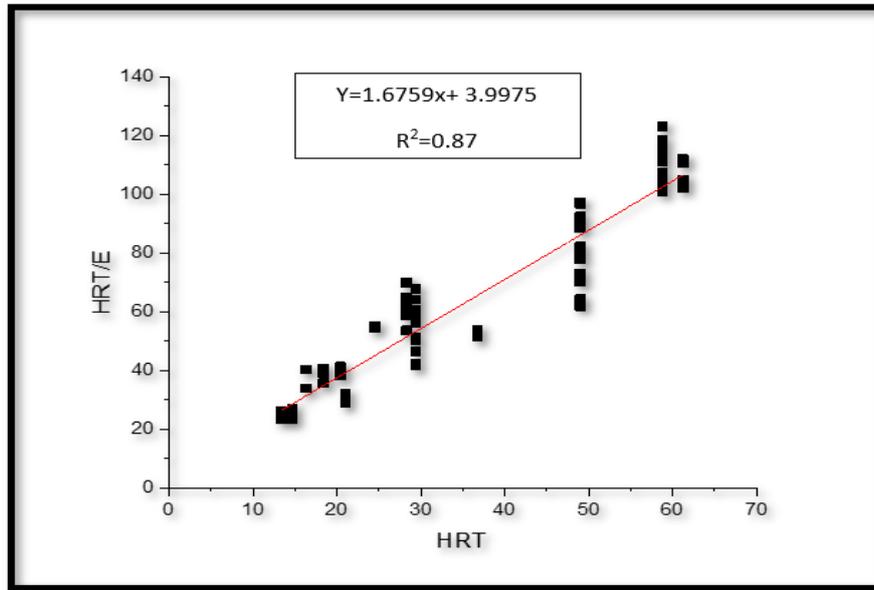**Grau's second-order kinetic model** :

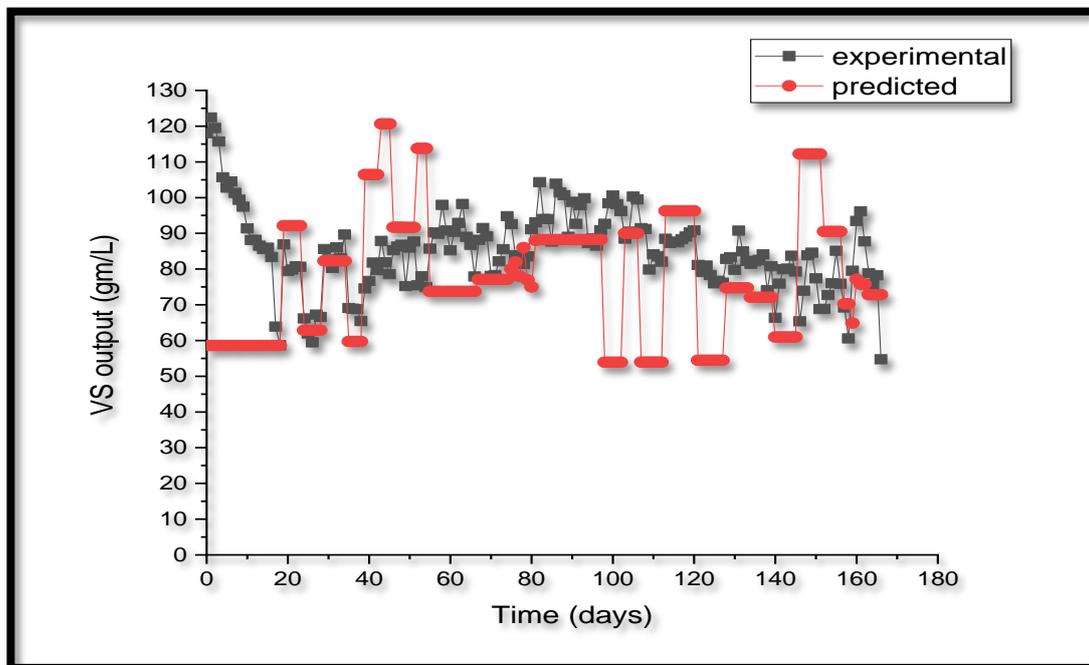Figure 18: Grau's Second-order kinetic model for Volatile Solids output

Figure 19: Experimental and predicted Volatile Solids output with Garu's second-order model

Grau's second-order kinetic model is also applied for effluent volatile solids prediction and kinetic constants a and b are 3.99 and 1.67 respectively with R^2 0.870

Equation 6 & 7 are used with kinetic constant U_{max}, K_B and a,b to determine predicted values of effluent Volatile solids concentration. Figures 27 & 29 show the predicted and experimental volatile solids concentration relationship.

In both the kinetic models, the highest variation is observed during the initial days of operation due to the acclimatization period and after the 80th day there is highest effluent volatile solids removal is predicted, this could be due to the substrate is fed with OLR 8 gmVS/L/d with highest total solids concentration can create operational deficiency and sudden change is substrate concentration reflects higher effluent volatile solids prediction during a particular period. A similar observation is also observed with predicted output Volatile Solids in Grau's second-order kinetic model. The experimental values are slightly inconsistent due to variations in substrate composition and characteristics with the same HRT and same OLR as taken to consider the real-time application of substrate

4.4 Prediction modelling using Artificial Neural Network (ANN)

Prediction modelling is a very important tool for the performance of anaerobic digesters in field. In real-time application of the anaerobic co-digestion process, the composition and characteristics of OFMSW and bio-flocculated sludge from SST vary from time to time and season to season. 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, the operator can control different parameters to achieve an uninterrupted anaerobic co-digestion process. To develop a prediction model using ANN total of 102 days (out of more than 200 days) of data is utilized from lab scale semi-continuous flow anaerobic co-digestion process when methane gas production is observed with different OLRs.

%TS, OLR (gmVS/L/d), pH, VFA/Alkalinity ratio and HRT(days) are considered input variables and methane yield (L/kgVS$_{removed}$) and %VS$_{removed}$ are output variables for the development of the prediction model. **%**TS represent the total mass of solids including both organic and inorganic fractions in a given influent substrate where organic fraction contributes to methane generation. Measuring organic matter in the substrate is the most significant aspect

of analysis of solids. The OLR is the most crucial parameter for the effective operation of the anaerobic reactor. OLR ranges in this study are 2,3,4,5,6,8 and 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 Organic Loading Rate (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. The methanogenesis phase is achieved when pH stabilises between 6.5 to 8.5. VFA concentration must be managed in the reactor for optimal treatment efficiency and methane yield. The ability of the substrate to neutralise acids is known as its alkalinity in the digester. VFA/Alkalinity ratio offers a CO_2 buffering capability for methane generation in addition to pH management. In this study, the VFA/Alkalinity ratio ranges from 0.1 to 0.5 during the study period. In the present study, the experiment is performed for semi-continuous flow only and HRTs 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. Maximising 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. The anaerobic co-digestion process helps to reduce the pollution load and effective conversion of biomass into energy before being disposed-off in the environment.

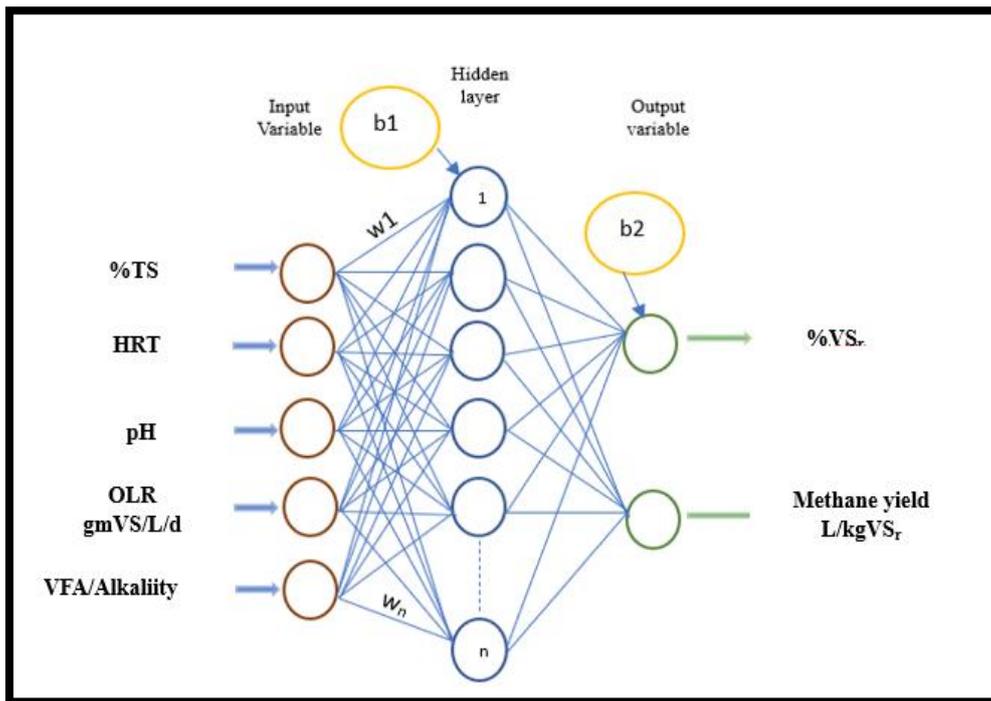### 4.4.1 ANN-based prediction model using the fitting application

Figure 20: Architecture of Artificial Neural Network

A Feed-Forward Neural Network (fitnet) is used for the development of a prediction model. The architecture of a neural network consists of five variables in input layers, one hidden layer with n numbers of hidden neurons and two variables in one output layer. A bias neuron is present in the input and hidden layers, providing stability to each neuron with constant activity (Figure 30).
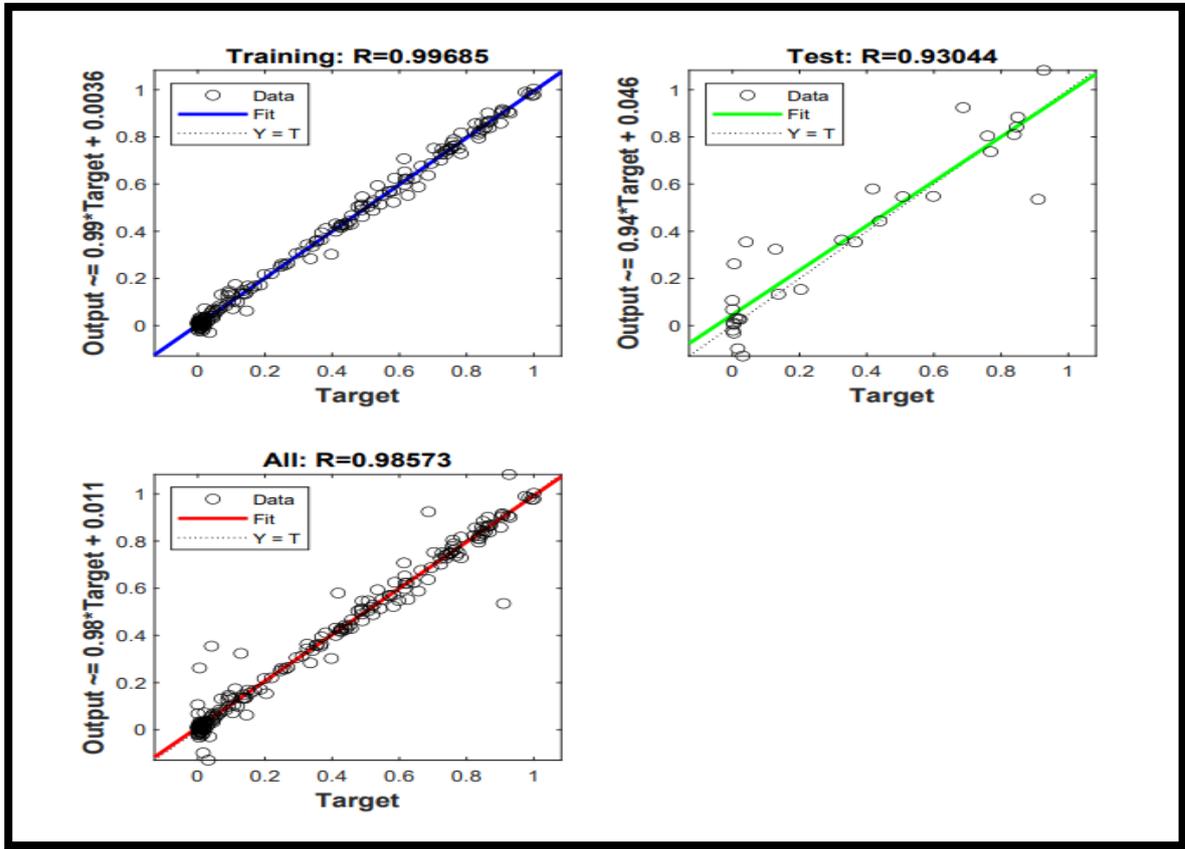
Figure 21: ANN performance model with training function Bayesian Regularization

Figure 31 presents the performance of the neural network using a regression curve for the training function Bayesian Regularization which uses the sigmoidal activation function (Olden & Jackson, 2002). ANN architecture has been assessed based on the Mean Squared Error (MSE) (Equation 15) and correlation coefficient (R) (Equation 18). According to equations 16 and Equation 17, the Mean Absolute Error (MAE) and Mean Absolute Percentage Error (MAPE) are computed.

Mean Squared Error (MSE)$=\frac{1}{x}\sum_{i=1}^{x}(P_i - E_i)^2$ Equation 15

Mean Absolute Error (MAE) $=\frac{1}{x}\sum_{i=1}^{x}\left|E_i - P_i\right|$ Equation 16

Mean Absolute Percentage Error (MAPE) $=\frac{1}{x}\sum_{i=1}^{x}\left|\frac{E_i - P_i}{E_i}\right| X\ 100\%$ Equation 17

$R = \dfrac{\sum_{i=1}^{x}(P_i - Pi)(E_i - Ei)}{[\sqrt{\sum_{i=1}^{x}(E_i - E_i)^2}][\sqrt{\sum_{i=1}^{x}(P_i - P_i)^2}]}$ Equation 18

Where,

x = number of datasets

E_i = experimental data

$\overline{E}_i$ = mean of experimental data

P_i= Predicted data output

$\overline{P}_i$= mean of predicted data output

R= coefficient of correlation

Annexures 2 & 3 show the lowest MAE, MAPE, MSE values and R applied for several training algorithms using various numbers of hidden neurons for the response of a trained ANN model for %VS$_{removal}$ and methane(L/kgVS$_{removed}$). The Feed Forward Neural Network with Bayesian Regularisation (BR training algorithm provides the best R-value (0.986) for the %VS$_{removal}$ at 17 hidden neurons, the lowest MAE value of 0.419, the lowest MAPE value of 0.006 and the lowest MSE value of 0.697. Furthermore, in a neural network trained with 2 to 20, the MAPE error has never surpassed 10% when using the BR training algorithm. The Feed Forward Neural Network with tan-sigmoid (fitnet) is trained using the Bayesian regularisation (BR) approach neural network trained with the BR training algorithm and 19 hidden neurons, resulting in the greatest R-value of 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. BR and LM training algorithms produce effective ANN models with the lowest MAE, MAPE and MAE values. Neural Network architecture 5-19-2 shows the correlation between experimental and predicted data for methane yield with R^2 0.95(Figure 28). Furthermore, %VS$_{removal}$ with network architecture 5-17-2 shows R^2 0.98 for the experimental and predicted data in Figure 29.
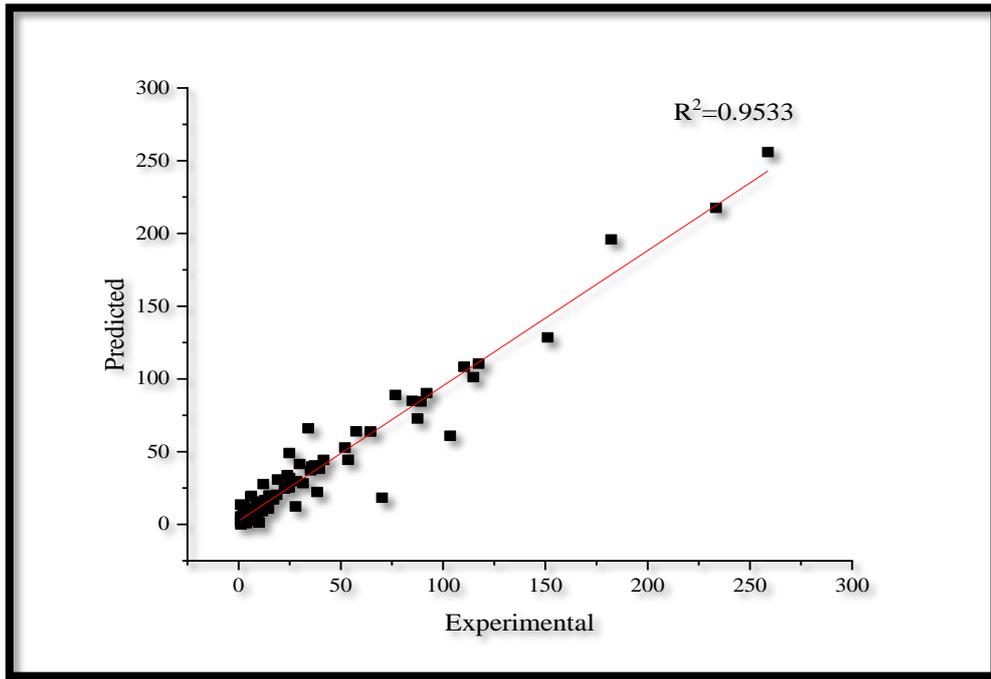
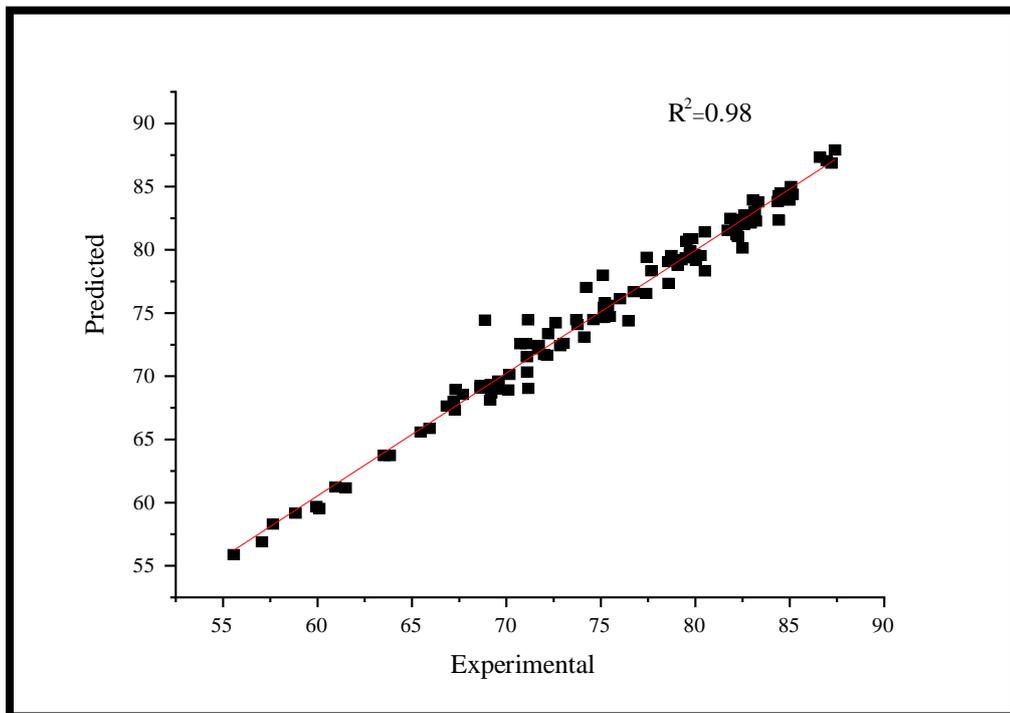Figure 22: Correlation between experimental and predicted methane yield (L/kgVS$_{removed}$)

Figure 23: Correlation between experimental and predicted %VS$_{removed}$

4.4.2 Development of prediction model using Feed Forward Back Propagation Neural Network (FFBP-NN)

ANN study is carried out with MATLAB to implement the Feed Forward Back-Propagation algorithm with training function Levenberg Marquardt (LM), Bayesian Regularization (BR) and transfer function tansig and logsig. It is studied in different literature that compared to other training algorithms, Levenberg Marquardt (LM)and Bayesian Regularization (BR) have continuously scored higher in terms of providing the greatest performances in the development of the ANN model (W. Y. Chen et al,2022).

In the present study, FFBP-NN with training function LM & BR shows compatible results with tansig compared to the logsig transfer function. Annexures 4 & 5 show the developed neural network model with training functions BR and LM for transfer functions tansig with different hidden neurons. The graphical representation of the FFBP-NN training and test results for this anaerobic reactor is shown in Figure 30.
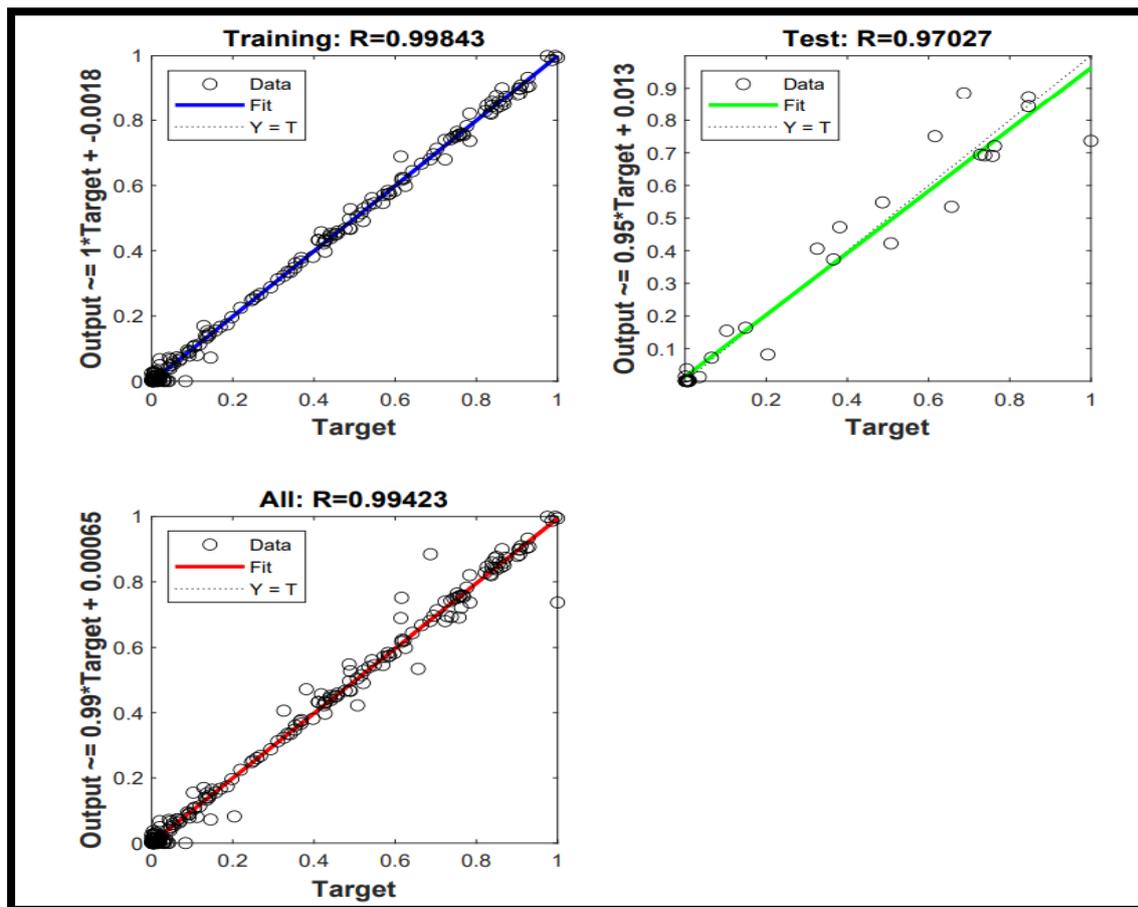

Figure 30 Graphical representation of FFBP-NN using Bayesian Regularization

It shows that the best developed FFBP-NN model with Bayesian Regularization training function and sigmoid transfer function with lowest MSE, MAE, MAPE and higher R-value for %$VS_{removal}$ and Methane yield (L/kg$VS_{removed}$) is for 14 hidden neurons with R 0.98.

Figure 31 (A) and (B) show the regression results of the plot between the experimental and predicted data using an FFBP-NN model for %$VS_{removal}$ and methane yield (L/kg$VS_{removed}$), respectively with BR training function and tansig transfer function for 5 input variable with one hidden layer of 14 neurons and 2 output variable. Experimental output simulated with ANN model with R^2 0.97 and 0.96 respectively for VS removal and methane yield.
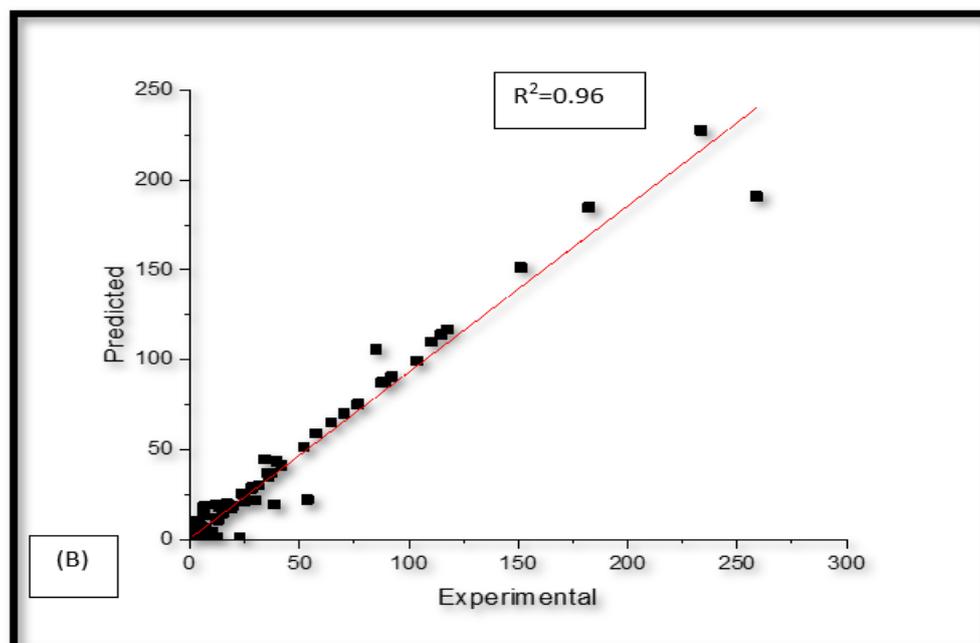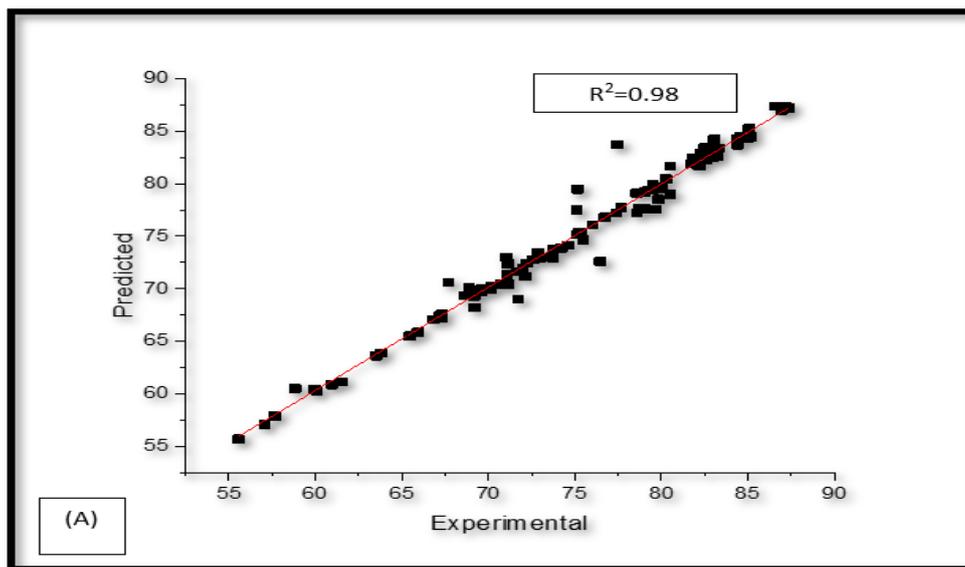
Figure 31 :(A) Correlation between Experimental and Predicted FFBP-NN of %VS$_{removal}$, (B) Correlation between Experimental and Predicted FFBP-NN Methane yield (L/kgVS$_{removed}$)

**4.4.2.1 Relative importance of Input variable for anaerobic co-digestion process**

The Feed Forward Back-Propagation Neural Network with training function Levenberg Marquardt (LM), Bayesian Regularization (BR) and transfer function tansig and logsig are applied for semi-continuous flow anaerobic co-digestion experimental data. Here, we summarise the significance of input and output variables using the connection weight approach for a feedforward back-propagation 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 co-digestion process of OFMSW and bio-flocculated sludge is significantly concerned with %TS of the input substrate, followed by HRT and OLR. The factors that affect methane yield are %TS > pH > VFA/Alkalinity ratio > OLR (gmVS/L/d) > HRT (Table 11).

Table 4: Relative Importance of input variable using Connection Weight approach

Output variable	Input variable	Calculated 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 model using ANN-PSO

In the present study, the Feed-Forward Neural Network is contrasted with the robust stochastic optimisation 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 optimise 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 34 shows how the MSE varies depending on swarm size and the number of neurons. To track the MSE fluctuation, a total of 2000 iterations have been completed. The MSE is 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 0.84. The computed R-value shows that the methane generated from the experimental ACoD of OFMSW and bio-flocculated sludge from SST is near the projected value of the biogas from the ANN-PSO algorithm.
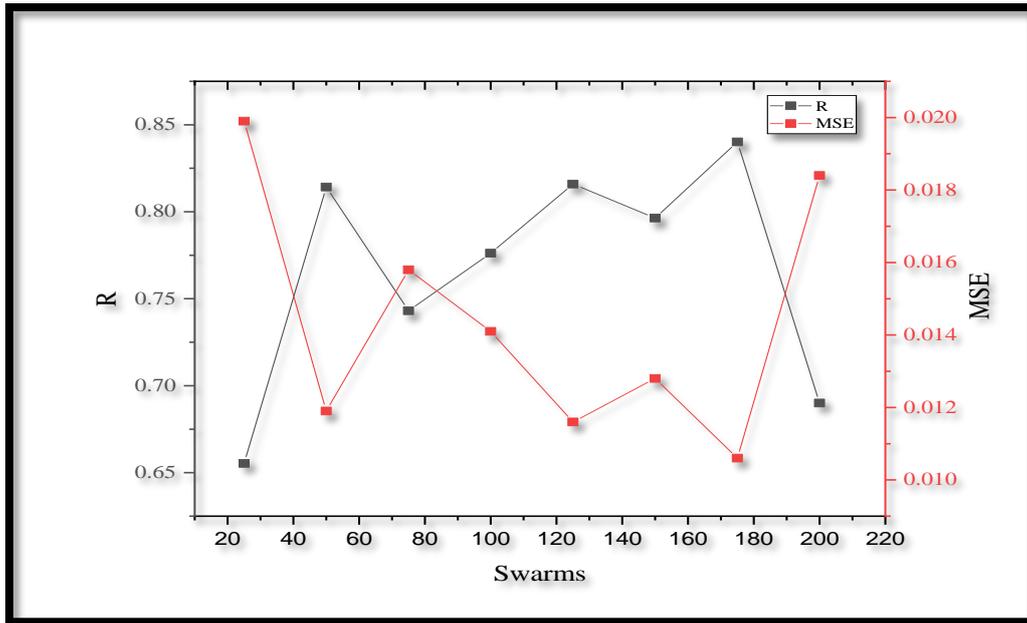
(A)
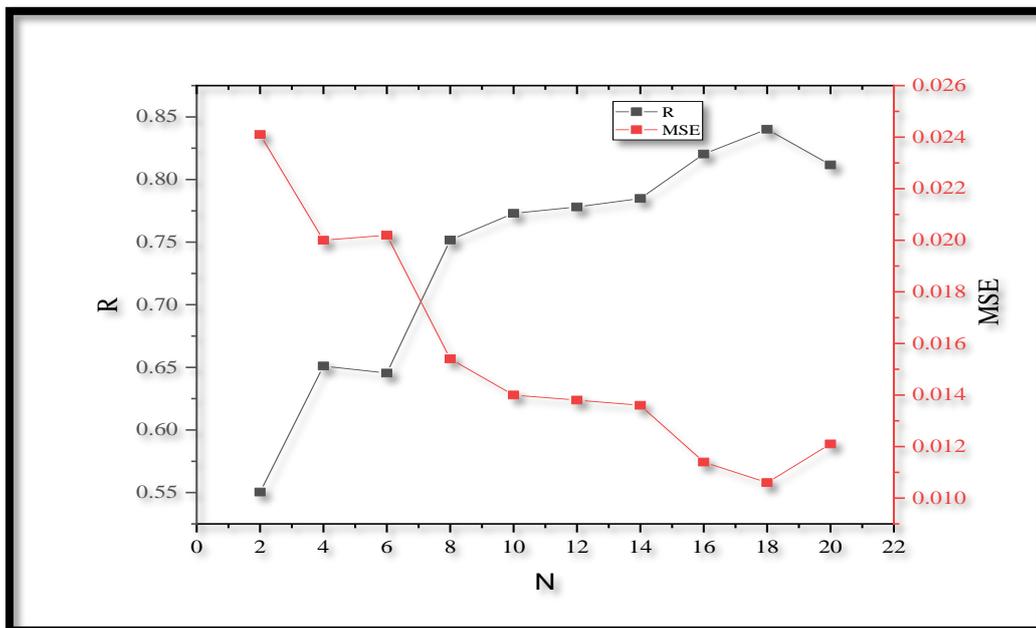
(B)

Figure 24: (A) ANN-PSO model for different numbers of Swarm size, (B) ANN-PSO model performance for different numbers of Neurons
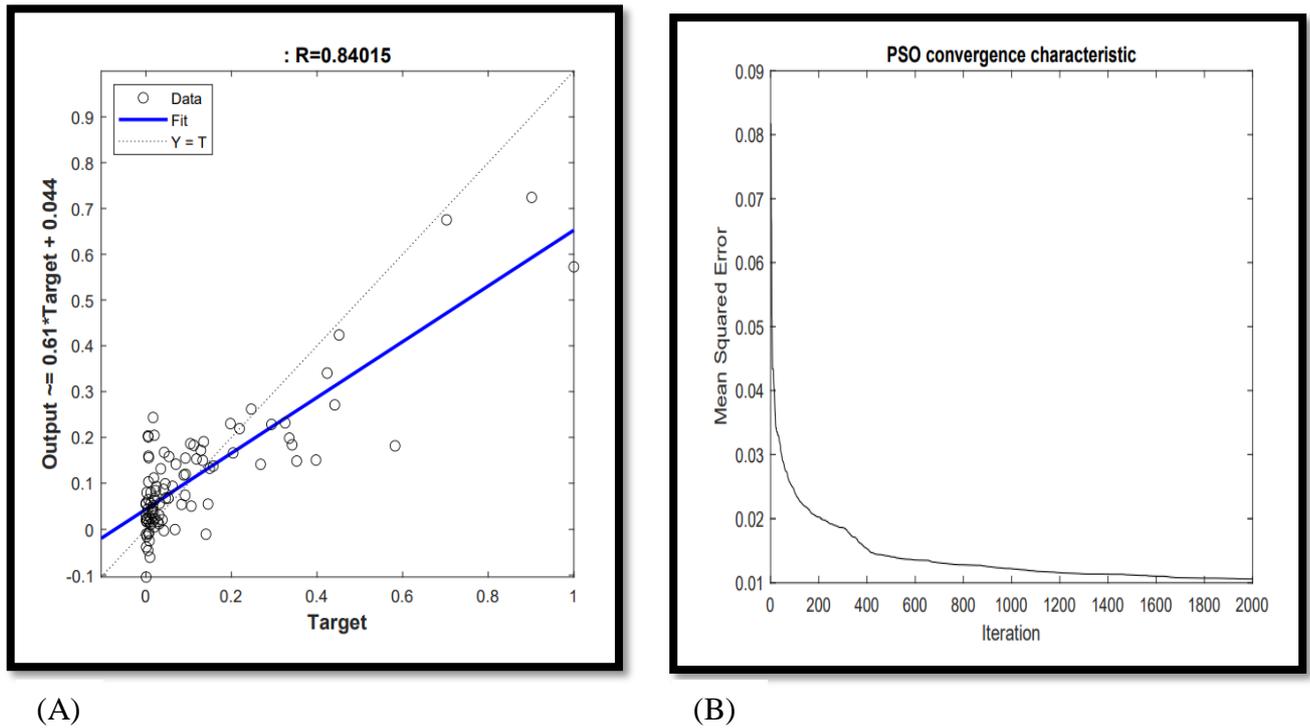
(A)   (B)

Figure 25: ANN-PSO (A) regression (B)convergence

Figure 32 depicts experimental and predicted methane yield using ANN-PSO with R^2 0.80.
The model accuracy shows that this model can help predict accurate methane yield for
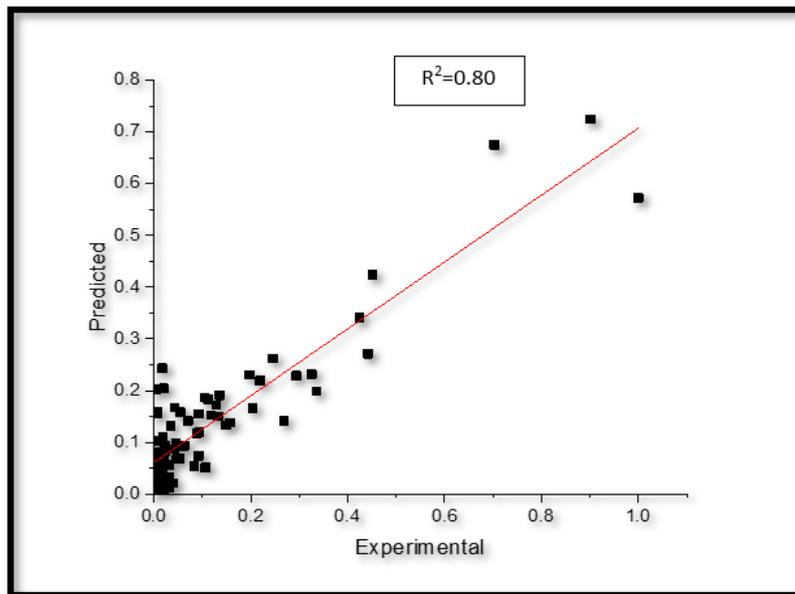unknown input variables.

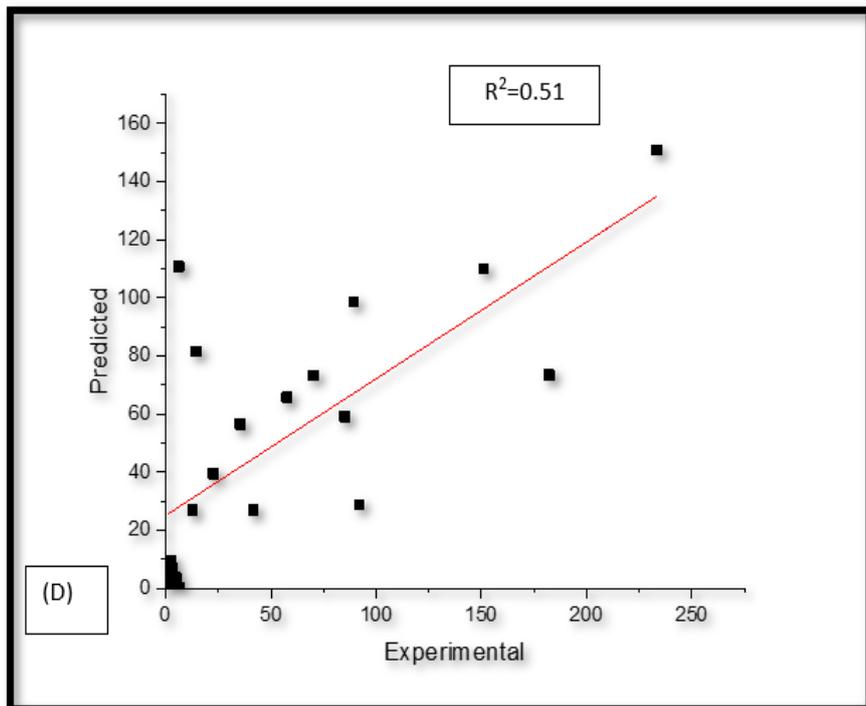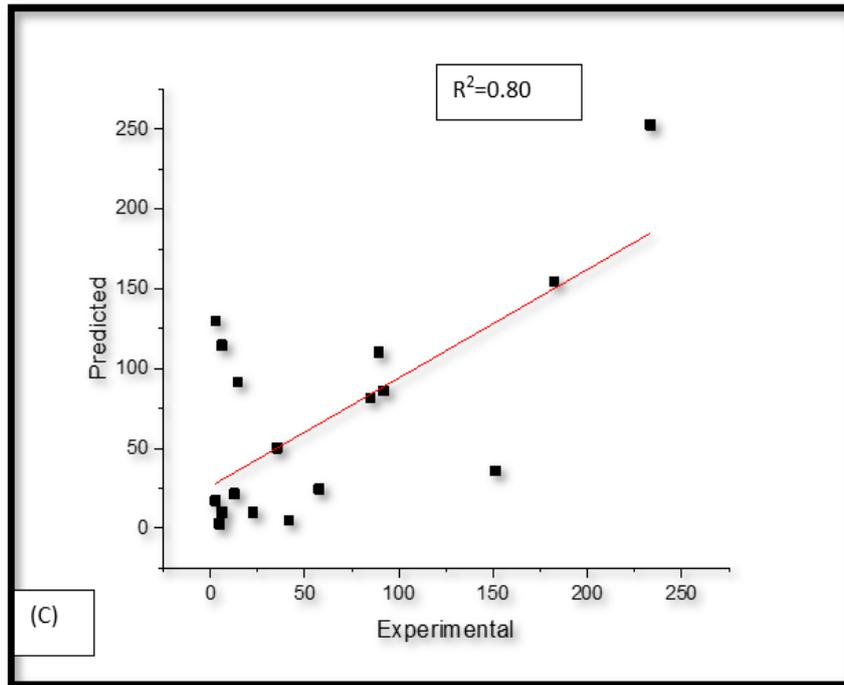Figure 26: Experimental and predicted methane yield using ANN-PSO

4.4.4 Prediction model using ANFIS

Semi-continuous flow anaerobic reactor experimental data are used to develop a prediction model using ANFIS. 75% of the data are used for training the model and 25% data are used for testing the model. In the present study, the ANFIS network is trained with five different types of membership functions (MFs) Triangular shape, Trapezoidal type, Gaussian type, Generalized bell-shaped, and Gauss2mf type. The efficiency of the development of the prediction model of each membership function is tabulated in Table 14 by using the MAPE and R^2 values of network performance. Gauss2mf type membership function with minimum MAPE value has the minimum error between predicted and experimental values and the maximum R^2 value can predict a more accurate model compared to the other four types of membership functions. The trained network with different membership functions and 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 has a maximum R^2 0.90 (Table 14) value for experimental testing data and predicted test data is considered the best membership function for the development of a prediction model for anaerobic co-digestion OFMSW and bio-flocculated sludge using ANFIS. Figure 33 shows model accuracy using experimental and predicted data with different membership functions. Gauss2mf (gaussian-2 membership function) has two adjustable parameters (i) centre and (ii) spread. The Centre determines the centre 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 a Gaussian-like distribution.

Table 12: ANFIS model development using different membership functions for methane yield

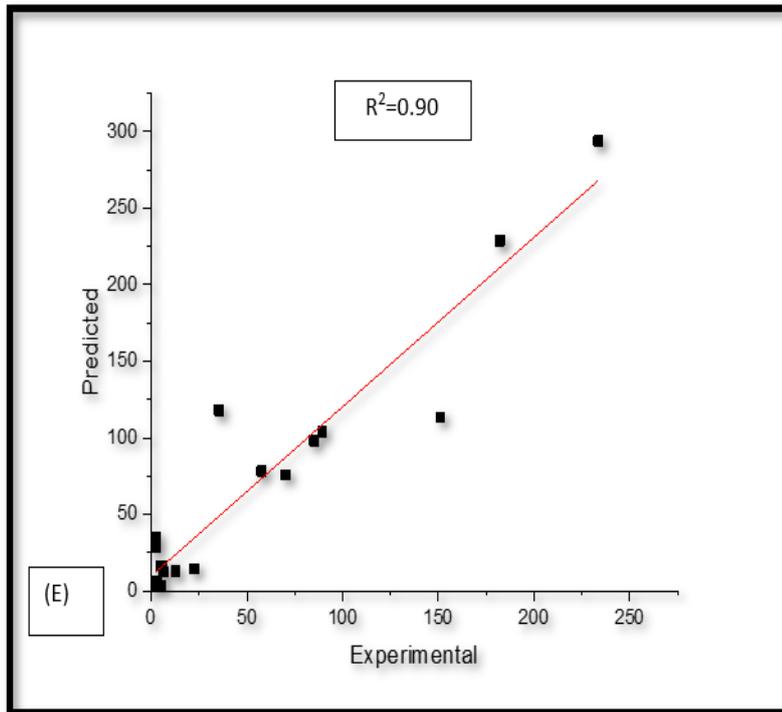| Membership function		Triangular shaped	Trapezoidal-shaped	Gaussian type	Generalized bell-shaped	Gauss2mf type
MAPE	Training	0.055	0.093	0.085241	0.017223	0.030273
	Testing	5.46	4.707699	2.156461	7.055921	2.448057
	All	1.43	1.271061	0.614064	1.814337	0.647579
R^2	Testing	0.65	0.47	0.79	0.51	0.90

(C)

(D)
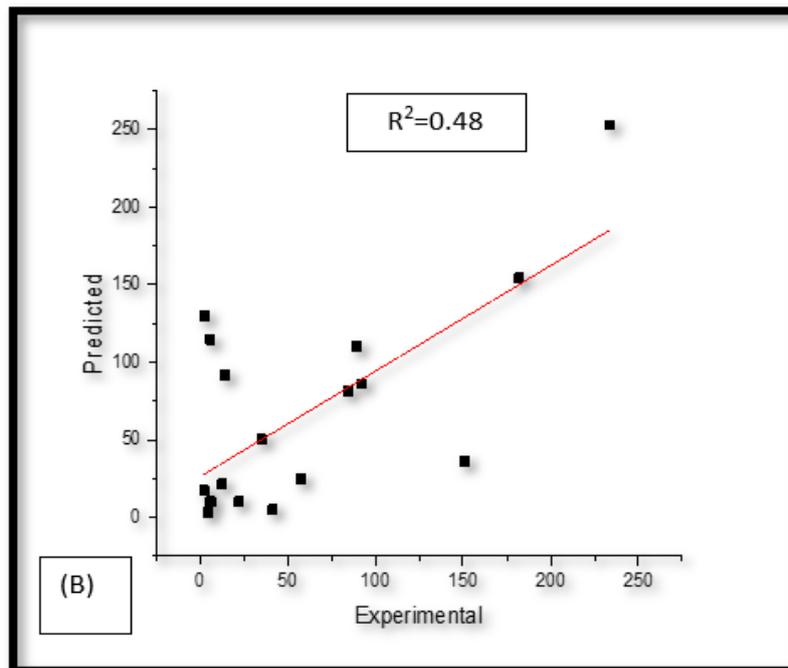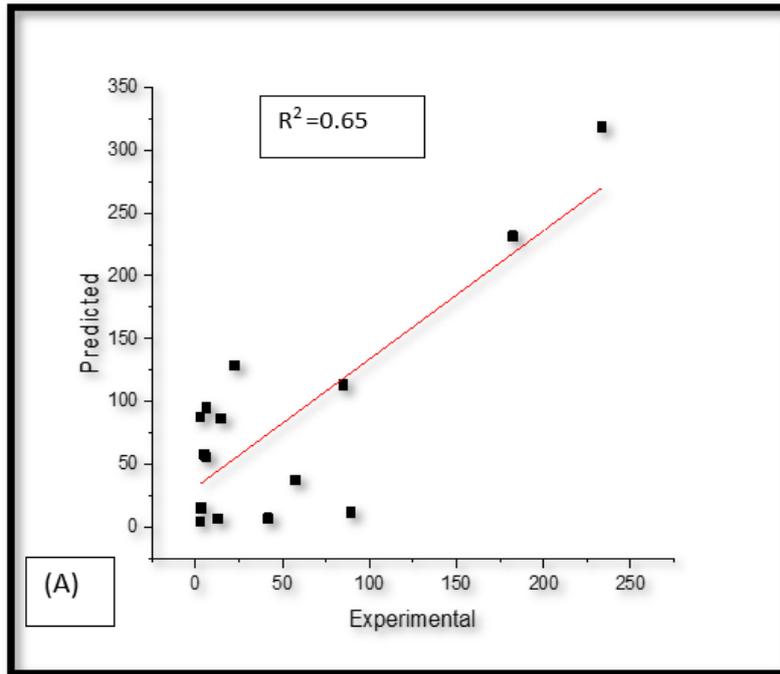
Figure 27: Co-relation between experimental and predicted ANFIS model data using different membership functions for methane yield using (A)trimf (B)trapmf (C) gaussmf (D)gbell (E)gauss2mf

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. To investigate microbial community, composition and dynamics in anaerobic digestion, Amplicon sequencing methods targeting the 16S rRNA gene have been extensively used.

4.5.1 Taxonomy composition analysis

During the pre-methanogenesis phase, the analysis shows the most abundant present phyla are Firmicutes (96.58%) and Proteobacteria (2.34%). These are the most common phyla responsible for fermentation of greater than 90% of the abundance found in the present study. These are reported mainly involved in the fermentation of complex polysaccharides. (Bareither et al., 2013; Cardinali-Rezende et al.,2009). Firmicutes are the most prevalent in co-digesting various combinations of wastes from restaurants, households and slaughterhouses. The microbial community of an anaerobic digester fed with a variety of feedstock demonstrates that many dominant populations belong to phyla Proteobacteria. within the phylum, the presence of Clostridia (69.09%), Bacilli (24.48%) and Gammaproteobacteria (1.83%) classes are majority available. Most of the bacteria belonging to the Firmicutes phylum are syntrophic bacteria that are often detected in ASP and anaerobic digesters having the capacity to degrade various VFAs (Grecia Penna et al., 2011). The class of Clostridia is well-known in fermenters. The majority of Clostridia in the anaerobic digestion is associated with a high rate of hydrolysis and VFA fermentation that occurred in the anaerobic digester.

Lachnospiracea, Lactobacillacea and Clostridiaceae are the dominant family of Clostridia and Bacilli class respectively for the fermentation process of anaerobic digestion. They performed the acidogenic process at the second stage and produced VFA, CO_2 and H_2 (Guo et al., 2015). Lachnospiraceae have the potential to metabolize the polysaccharides, aromatics and proteins that compose lignocellulose. They transform low-cost, sustainable, lignocellulosic feedstocks (i.e., forestry, agricultural, and municipal wastes) into value-added biochemicals (Zaplana et al., 2023). Lacnospiraceae break down polysaccharides, convert them into ethanol and propanol and also release CO_2 and H_2 gases and generate butyrate, propionate, lactate, acetate and 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 CO_2 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 as Lachnospirales of the Lahnospiraceas family and from Firmicutes Phylum (Jayanama et al., 2022) while the 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
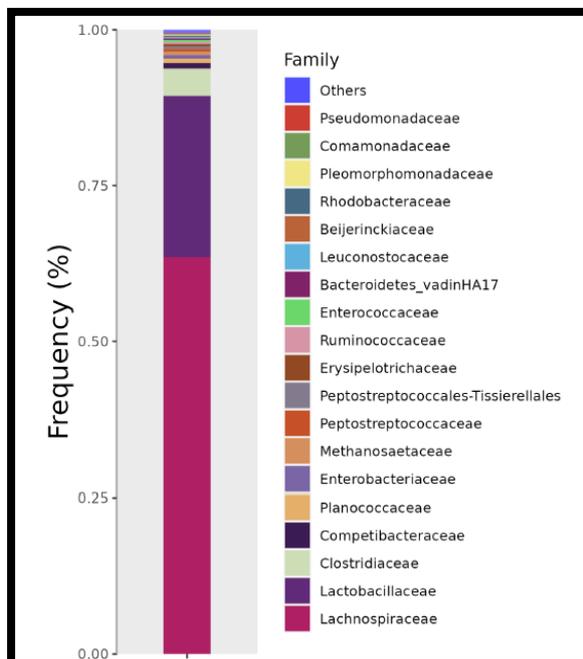
hydrolysis and acidogenesis which indicates that the hydrolysis and acidogenesis proceeded throughout anaerobic digestion (Meng et al., 2018).
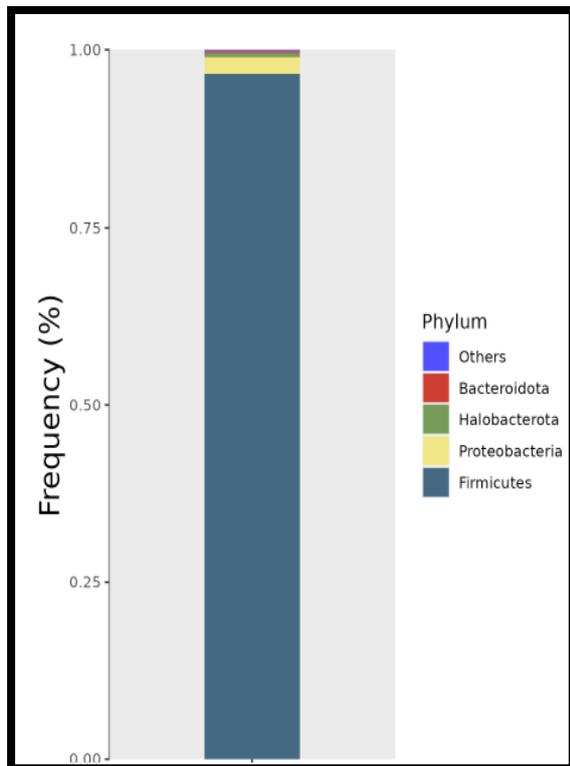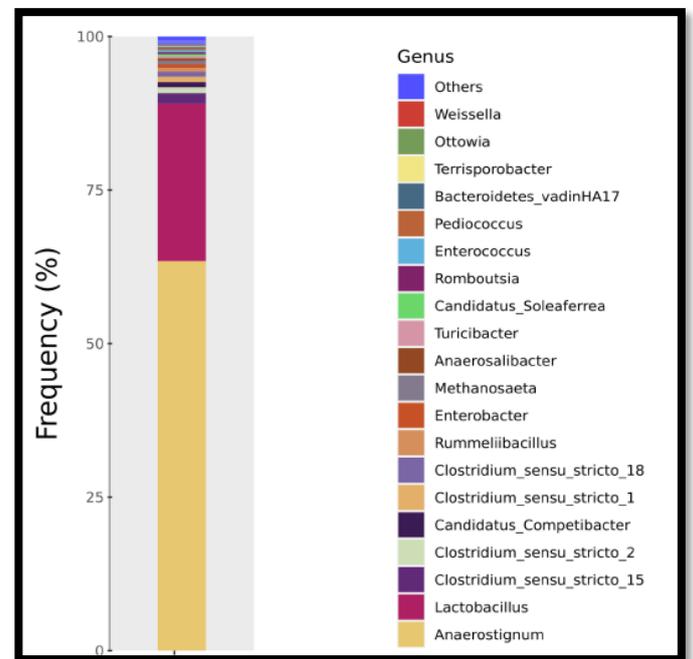

(A) Phylum
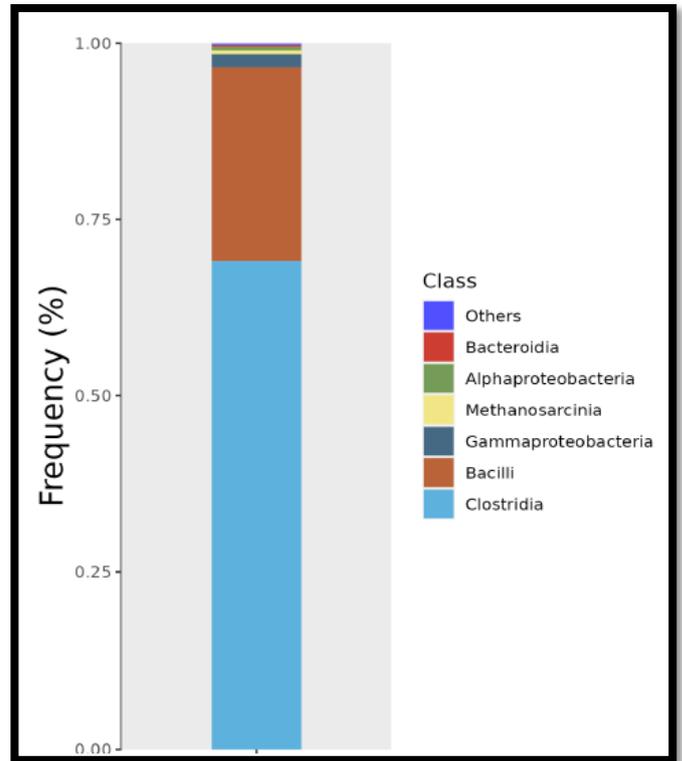
(B) Class

(C) Family

(D) Genus

Figure 28: Abundance of bacterial community at (A) Phylum level (B) Class level (C) Family level (D) Genus level