INTERNATIONAL JOURNAL of RENEWABLE ENERGY RESEARCH
S. Mothe et al., Vol.14, No.1, March, 2024

Anaerobic Co-Digestion of Rice Straw with Ternary Mixtures for Enhanced Methane Production

Sagarika Mothe[1], K. Bella[1], M. J Sukesh[2], B. Gopal[1], P. Venkateswara Rao[1*], Pilli Sridhar [1]

[1]Water and Environment Division, Department of Civil Engineering, National Institute of Technology - Warangal, Telangana, India.
[2]Sri Rajeswari Institute of Technology, Porddatur, Andhra Pradesh, India

smothe@student.nitw.ac.in, bellz@student.nitw.ac.in, bg72005@student.nitw.ac.in, m.sukhesh@gmail.com, pvenku@nitw.ac.in, srenitw@nitw.ac.in

Corresponding author, Email: pvenku@.nitw.ac.in; srenitw@nitw.ac.in

Received: 27.12.2022 Accepted:03.03.2023

Abstract: A high carbon-to-nitrogen ratio makes rice straw unsuitable for mono-digesting. This study investigates the effect of various proportions of total solids (15%, 20%, 25%, 30%) on the biodegradability of rice straw during co-digestion with nitrogen-rich substrates, such as food waste, cow manure, sewage sludge, and chicken manure. As a result of co-digestion, the process stability and volumetric yields of biogas were significantly increased. The mixture of RS+CM+ChM (rice straw + cow manure + chicken manure) (F) with a solids ratio of 20% reported a higher biogas yield of 442 mL/g-VS with a higher methane yield of 55.88%, followed by the mixture of RS+SS+ChM (rice straw + sewage sludge + chicken manure) (E) with 408 mL/g-VS with a solid's ratio of 20%. In mixtures of rice straw, cow manure, and food waste biogas production is lowest due to the accumulation of volatile fatty acids from the co-substrates that can be easily biodegraded. The rice straw mixed with sewage sludge and chicken manure consistently performs better than all other mixtures at all four total solids contents. The Modified Gompertz model fitted the experimental values well and noticed a rise in lag phase times ranging from 1.28 to 10.04 days in the case of RS+SS+CM (D) mixture. From the results of the experiments, the maximum production (P_{max}) of biogas obtained from RS+SS+ChM (rice straw + sewage sludge + chicken manure) (E) at 20% TS was 470.01 mL/g-VS and RS+CM+ChM (rice straw + cow manure + chicken manure) (F) at 20% TS was 447.47 mL/g-VS. Thus, ternary mixtures with rice straw enhance digestion and biogas production.

Keywords: anaerobic digestion, co-digestion, rice straw, methane, biogas.

1. Introduction

India is one of the agrarian countries where a major portion of land is utilised for farming and rice lies as the second most important crop. The total harvested has come to around 60 million acres yielding approximately 760 Mt (metric tons) per year [1]. Cultivation of rice crops delivers two farming residues which are rice husk (RH) and rice straw (RS). In India alone, the generation of RS residues is around 60.8Mt annually [2]. RS is mostly used by farmers for roof thatching and as animal fodder [3]. Even though there are several utilisation techniques available for RS, like mulching, electricity generation, composting, biogas production, as ruminant feed, and use in the production of composite materials, the RS is usually burned openly in fields, which is a common practice across India and around the world [3]. Conventionally, the open field burning of RS is one of the regular practices adopted in all large rice-producing countries, including India, China, Thailand, Indonesia, etc. In India, it is estimated that 23% of RS remains or is burned, and this open burning of RS contributes 0.05% to the emission of greenhouse gases, a threat to the atmosphere and climate [4]. This practice not only results in the degradation of the air quality by discharging toxic air pollutants but is also liable for injurious health effects [5, 6]. Most farmers prefer open burning of RS despite its negative consequences since it involves no cost, simplifies tillage, and reduces weeds in upcoming crops. However, RS burning emits gases such as carbon dioxide (CO_2), methane (CH_4), carbon monoxide (CO), nitrous oxide (N_2O), oxides of nitrogen (NO_x), hydrocarbons, etc. [7]. It is one of the major causes of air pollution in many cities close to farming fields [8]. The surplus residues can be utilised for energy recovery with minimal environmental consequences. Further, efforts are required to consider the application of the residue as a source, in turn implementing agroecosystem sustainability [9].

Anaerobic digestion (AD) is one of the promising technologies to utilise RS as a source where its biochemical composition reflects a good substrate for AD. AD is a biological practice where various microbes alter the complex organic substance into simple and stable end output without oxygen. A benefit of the process is that it produces biogas and digestate, where biogas can be used as renewable energy and digestate as a fertiliser [10]. The AD of solid wastes is progressing rapidly owing to its advantages over other processes in the same segment, particularly high solid AD, which is becoming the main concern in waste management worldwide. This technique sounds to be reliable and assuring for the treatment of solid organic substrates like RS [11], wheat straw [12], corn stover [13], sugarcane waste [14], rice husk [15], biochar [16], etc. Depending on total solids content (TS), there are two main types of AD processes, i.e., liquid (wet, TS <10%) and solid-state (semi-dry, 10% < TS < 20%; dry, TS > 20%) AD. Qian et al. [11] have suggested that substrates in their original form without dilutions have gained increased results in biogas yield and production rate. Furthermore, solid-state anaerobic digestion (SS-AD) has several advantages, including greater volumetric methane productivity, reduced heating energy requirements, less wastewater generation, and a digestate with less moisture that is easy to employ and less investment cost, which makes them particularly attractive to developing countries [17,18]. The system has, however, several shortcomings, including insufficiency of mixing, insufficient mass transfer, and instabilities due to high organic loading, which may lead to the accumulation of inhibitors, such as ammonia and volatile fatty acids (VFAs) [18]. To overcome these limitations for RS, some techniques have been put ahead, such as co-digestion, selection of appropriate inoculum, adopting material pre-treatment, and maintaining the reactor temperature. Among the above, Co-digestion is presumed to be the most effective for nutritional addition compared to pre-treatment [19]. Co-digestion refers to the simultaneous AD of multiple substrates for better synergism. As RS is a high C/N ratio feedstock, co-digesting with nitrogen-rich substrates not only balances the improper C/N ratio but also improves the stability of AD. Consequently, this may lead to improved methane yield while reducing the inhibitory effects of mono-digestion and rising utilisation rates.

Anaerobic co-digestion of RS with several co-substrates, including cow manure [20], sewage sludge [21], activated sludge [22], food waste [23], municipal solid waste [24], pig manure [25], pig urine [26], chicken manure [27], and industrial wastes [28] was evaluated to enhance biogas production. Some studies on optimum TS content for the anaerobic co-digestion of RS have already been done. Qian et al. [11] experimented on co-digestion of RS with pig manure at 15% TS has improved the biogas productivity by 25% due to increased synergism of digestion. Neutralising the VFAs with biochar, Liu et al. [16] studied the co-digestion of food waste, and sewage sludge with biogas residue biochar at a feedstock ratio of 1:1 produced the highest daily methane yield of 432.2 mL/g-VS. Kaushal et al. [29] studied the co-digestion of food waste, algae, and cow manure mixed with chicken and fish waste and produced a higher biogas production of 2244.58 mL when chicken waste was included. Co-digestion enhanced the hydrolysis waste degradation efficiency [29]. According to Rahmani et al. [12], several inoculum-to-food ratios (0.5, 1, 2, 2.5) have been experimented on concurrently at TS% (5, 7.5, 10, 12.5%). At TS 12.5%, a C/N ratio of 35 and a ratio of inoculum to food of 2 have improved results and degraded more than half of the substrate due to co-digestion synergy. During co-digestion of corn straw, food waste, and chicken manure at different VS ratios of food waste, Zhu et al. [30] investigated (corn straw and chicken manure) (with constant corn straw to chicken manure ratio of 3:1) as 8:2, 7:3, 4:6, 2:8, whereas, at 8:2 the highest amount of methane was produced, 125% higher than that of mono digestion. According to the literature on co-digestion, ternary combinations can increase the degradation of organic substrates, facilitate their hydrolysis, and produce additional methane. The recent state of art review by Mothe et al. [31] on AD of RS shows almost the binary combinations, suggesting the potential for ternary mixture AD for RS.

To optimise co-digestion, determining the optimal co-digestion mixture, determining the specific methane yield, and quantifying the parameters that analyse the stability of all combinations were crucial to determining the best co-digestion mixture. Hence, the objective of this study is to determine which combinations of RS and selected substrates are most effective at several total solid contents, including 15%, 20%, 25%, and 30%. Thus, the novelty of the current study is to explore the chances of utilizing locally available co-substrates for improving the biodegradability of RS with ternary mixtures at high solid content. In addition, the kinetic parameters (P_{max}, Q, and λ) obtained from the Modified Gompertz equation and the experimental results are studied.

Table 1: Recent Literature review of ternary mixtures

Substrates	Reactor type	Digestion conditions	Pre-treatment	Biogas/ methane yield	Remark	Reference
Biogas residue biochar, food waste, sewage sludge	Semi-continuous	Thermophilic (55 ± 1°C)	no	Highest daily methane yield of 432.2 mL/g-VS	Due to the basic nature of biochar and co-digestion	[50]
Food waste, algae, cow manure, chicken waste, fish waste	Batch	Mesophilic (35°C to 42°C)	Alkali pretreatment	Highest cumulative biogas produced of 2244.58 mL	Due to an increase in hydrolysis efficiency and increase in waste degradation efficiency	[29]
Wheat straw, food waste, cattle manure	Batch	Mesophilic study	no	80% more biogas production than mono digestion and 48% VS removal	Co-digestion synergism	[12]
Corn straw, food waste, chicken manure	Batch	Thermophilic (55 ± 2°C)	no	Methane efficiency of 125.3% than mono digestion	Co-digestion synergism	[30]

1. Materials and Methodology

1.1 Substrates and Inoculum

In the current study, the main substrate is RS, and the co-substrates chosen are food waste (FW), sewage sludge (SS), cow manure (CM), and chicken manure (ChM). RS was collected from farmlands in Kazipet of Warangal district, Telangana, India, cut to 1-2 cm size, and stored at room temperature. FW is leftovers from NIT Warangal hostel. It consists mostly of boiled rice, vegetables, and legumes dal. Sun-dried sewage sludge is obtained from the wastewater treatment plant at the NIT Warangal campus. CM was brought from agricultural lands near Kazipet, Warangal district, Telangana, India. ChM was brought from farms near kazipet, Warangal, and all the co-substrates were stored at 4°C. Inoculum is a liquid anaerobic digestate from the anaerobic plant installed at the NIT Warangal campus, which has been acclimated for five days at 37°C ± 2°C. The characteristics of substrate and co-substrates are summarised in Table 2.

Table 2: Characteristics of the substrates

Parameter	Rice straw[b]	Chicken Manure[b]	Cow Manure[b]	Food Waste[b]	Sewage sludge[b]
TS (%)	89.77 ± 0.40	50.04 ± 0.06	14.56 ± 0.52	47.01 ± 0.68	34.79 ± 0.05
VS (% TS[a])	85.27 ± 1.70	29.05 ± 0.02	76.87 ± 0.70	90.70 ± 1.24	69.50 ± 0.80
C (% TS[a])	37.82	14.14	30.02	42.36	22.7
N (% TS[a])	0.74	1.83	1.62	2.1	1.2
C/N	51.11	7.73	18.53	20.17	18.9
COD (g/l)	97.28 ± 0.90	69.12 ± 0.73	58.88 ± 0.60	10.24 ± 1.10	89.60 ± 0.95
Cellulose (% TS[a])	33.14 ± 1.15	NA	NA	NA	NA
Hemicellulose (% TS[a])	19.73 ± 1.28	NA	NA	NA	NA
Lignin (% TS[a])	13.1 ± 0.52	NA	NA	NA	NA

NA: not available.
[a] TS: Total Solids
VS: Volatile Solids
[b] values mentioned are means ± standard deviation (n = 3).

2. Materials and methods

2.1 Experimental Design and Setup

Batch reactors (glass bottles) were used for the biomethane potential (BMP) assay having a capacity of 120 mL. The reactor bottlenecks were tightened with a rubber cork and aluminium crimp with an attachment for collecting biogas after flushing with nitrogen in the head space. Reactors were manually shaken to mix the contents two times a day. Glass bottles were flushed with nitrogen gas before adding substrates and sealed with rubber septa and aluminium caps after pH measurements were taken. Three bottles each were kept for each mix and were controlled at a mesophilic range of 37°C ± 2°C for 65 days. To determine the gas composition, we measured the volume of gas every day with a leak-proof glass syringe and, after an eventual decrease in gas production, every alternate day.

The solid-state anaerobic co-digestion of RS has been studied to form six ternary mixtures as, i) RS+FW+CM (A), ii) RS+FW+ChM (B), iii) RS+FW+SS (C), iv) RS+SS+CM (D), v) RS+SS+ChM (E), vi) RS+CM+ChM (F) simultaneously at 4 different TS contents, i.e., 15%, 20%, 25%, and 30%. RS substrate, high in C/N ratio of around 50, was co-digested with complementary substrates with low C/N ratios, and all combinations were formulated to see a 25 C/N ratio, as suggested by [32]. The co-digestion experiments were carried out for 65 days.

2.2 Analytical Methods

The physical and chemical characteristics of substrates and co-substrates like pH, total solids and total volatile solids, and chemical oxygen demand were estimated as per the guidelines explained in "Standard Methods for the examination of water and wastewater" (APHA, 2005). The quantification of biogas was done by measuring the volume using a glass syringe. Gas chromatographic analysis of biogas (YL Instruments Model 6500) with a thermal conductivity detector and Porapak Q column of 15 feet in length was performed. The carrier gas was hydrogen gas, and the injection port, detector, and column oven temperatures were retained at 40℃, 100℃, and 50℃, respectively. The composition of biogas was analysed using a standard biogas mixture (51.60% carbon dioxide and 48.3% methane by volume). Carbon and nitrogen of substrates were analysed using elemental analysis (Euro EA Elemental Analyzer). Cellulose, hemicellulose, and lignin were measured using the approach outlined by Li et al. [33]. Volatile fatty acids are computed from the Nordmann method [34]. Liquid samples were centrifuged at 6000 rpm for 15 minutes at room temperature and filtered with a 0.22 µm membrane filter for VFA analysis.

2.3 Kinetic Study

The modified Gompertz model is one of the mostly employed models for simulating the experimental biogas production values in AD. The model can be used to derive the correlation between cumulative biogas production and fermentation time. The modified Gompertz model was used in this study to model biogas production values obtained for different co-digestion mixtures at various TS % ranges [35]. The model Equation (1) is as follows:

$$Y(t) = P_{max} \times \exp\{-\exp[\frac{Q \times e}{P_{max}}(\lambda - t) + 1]\} \quad \text{Eqn (1)}$$

where Y(t) is the cumulative biogas production at time t expressed in mL/g-VS, P_{max} is the maximum methane potential expressed in mL/g-VS, and Q is the biogas production potential expressed in mL/g-VS.d, λ is the lag phase time in days, and e is Euler's constant of value 2.7182. Statistical analysis to test the significance of results has done by ANOVA.

3. Results and Discussion

3.1 Substrates Characterisation

The physicochemical characteristics of the substrate (RS), co-substrates (FW, CM, ChM, SS), and inoculum are mentioned in Table 2. The characteristics like TS, VS, total carbon, total nitrogen, COD, cellulose, hemicellulose, and lignin were analysed prior to the experiment, whereas VFA, pH, and VS% were measured after 65 days of the experiment. The TS content (89.77%) of RS is comparatively higher than that of other substrates, and a similar range of values is reported by Ye et al [36]. The VS content in all substrates is reported above 60% except in ChM (29%). However, AD of ChM reported higher biogas production in earlier studies [27]. The typical chemical characteristics of plant biomass like lignin, cellulose, and hemicellulose of RS are 13.1%, 33.14%, and 19.73%, respectively. This falls under the range given by the Japan Institute of Energy (2002), which is 12% lignin, 25% hemicellulose, and 38% cellulose [37]. The C/N ratios of RS, FW, CM, ChM, SS were 51.11, 20.17, 18.53, 7.73 and, 18.9 (Table 2), respectively. RS possesses a higher C/N ratio, much higher than the optimum value (20-35) suggested for a stable AD process [32]. The co-substrates chosen have a relatively lower C/N ratio, which helps achieve the optimum range of C/N ratio for the mixture.

3.2 Biogas and Methane Production

The cumulative biogas production for various co-digestion mixtures of RS with dual combinations of FW, CM, ChM, and SS at different TS concentrations is illustrated in Figure 1. The biogas production values are normalised using VS content present in the mixtures. The maximum cumulative biogas production was observed as 442 mL/g-VS on the 35th day for the ternary mix F at a TS content of 20% with an average methane content of 55.38%. For E on the 40th day, a 408 mL/g-VS value was obtained at 20% TS content. Previous studies have shown increased biogas production when rice straw and nitrogen-rich substrates like chicken manure and cattle manure are co-digested. A study by Wang et al. [38] showed similar kinds of increased biogas production (343 mL/g-VS) values for mixtures comprising chicken manure and cattle manure digested with rice straw. A desirable C/N ratio may have assisted in increasing biogas production in the current study by mixing RS with CM and ChM. A similar yield of 383.5 mL/g-VS was observed by Li et al. [20] during AD of RS with CM. The biogas production at TS 25% and 30% are low compared to the other two TS contents (15% and 20%) in all the mixtures.

Next to CM and ChM, FW showed better compatibility with rice straw. In combinations of SS with RS, a maximum biogas yield of 408 mL/g-VS was obtained at 20% TS content. AD of low C/N ratio feedstock often leads to the release of NH_4-N in the system and causes direct inhibition of microbial activities [39]. Hence, the combination of RS with SS and CM served as an optimum mix for a stable AD process without causing ammonia inhibition.

As per some studies by Sasaki et al. [40, 41], carbon fibre textiles (CFT) have been reported to be effective in treating ammonia toxicity in wet AD systems. In the mixtures comprising SS and ChM, at four TS ranges of 15%, 20%, 25%, and 30%, the biogas production values obtained are 357.3, 408, 315.2, and 278 mL/g-VS, respectively. As for F, TS 20% outperformed among 4 ranges; however, all ranges attained results within 35 days with an average methane content of over 50%.

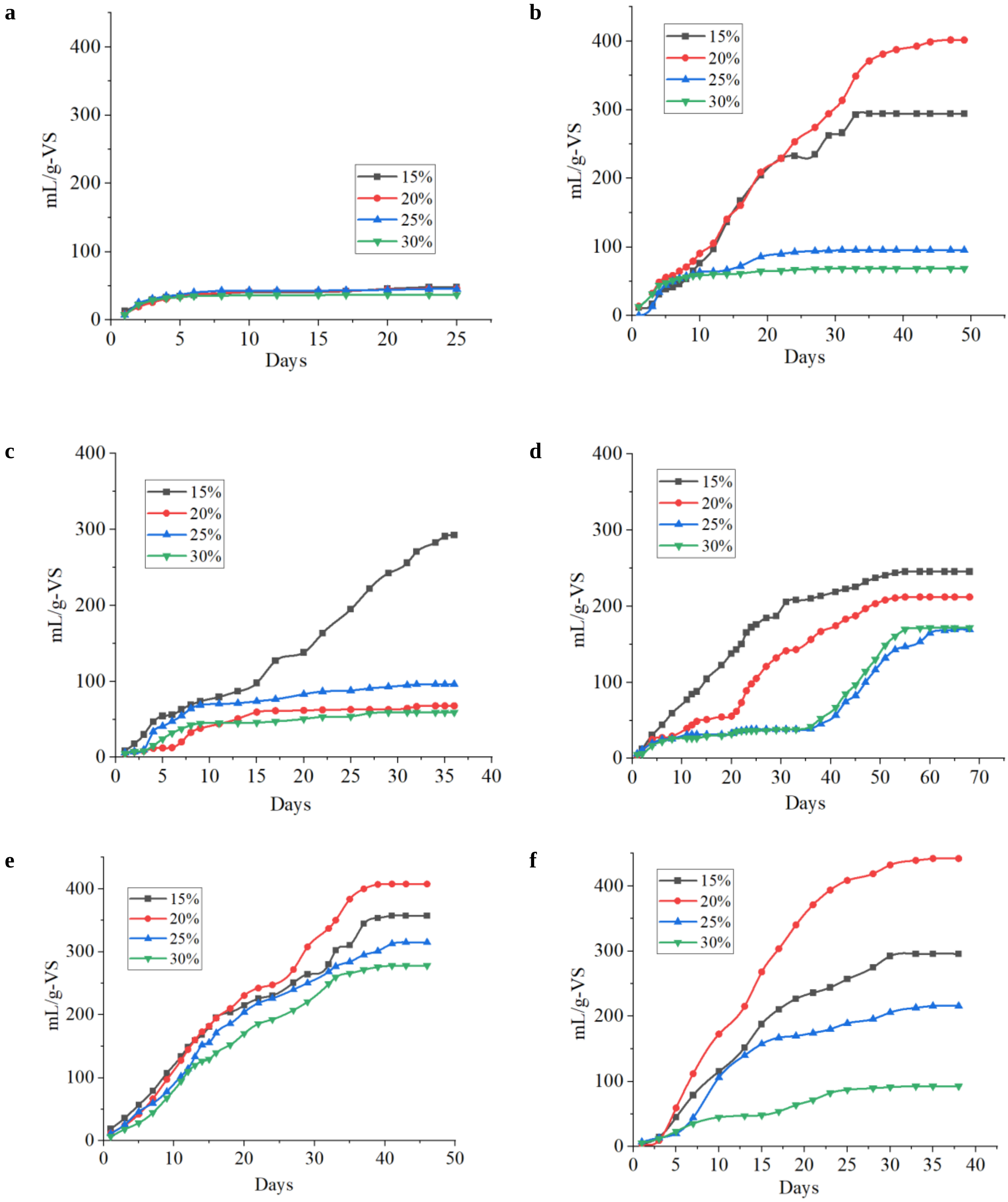


Figure 1: Graphs showing cumulative biogas yield in mixtures A, B, C, D, E, and F respectively.

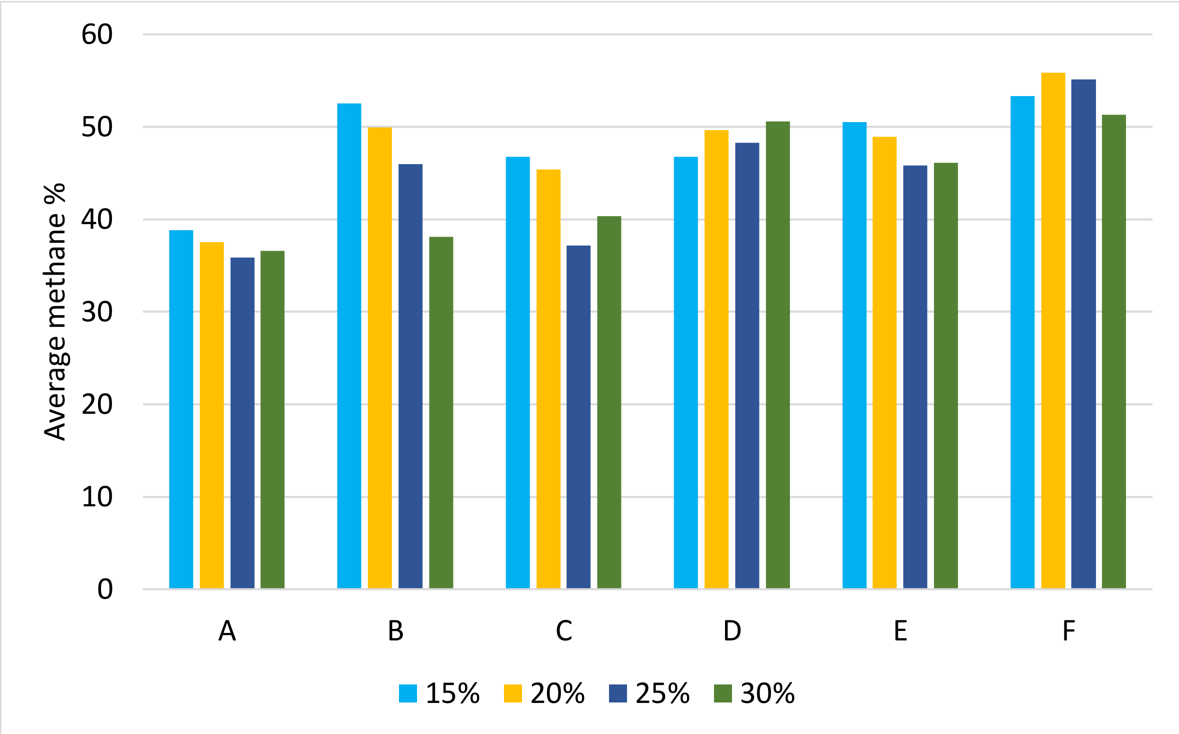


Figure 2: Average Methane content (%) in each mixture at all Total solid contents

The ternary mixture comprising RS with FW and SS showed the lowest biogas production value, i.e., 48.32 mL/g-VS. For other ternary mixtures having FW mixed with CM, and SS, the biogas production values obtained are comparatively more than that with SS, i.e., 245.39 mL/g-VS, respectively. Although the volatile fraction and moisture content in FW are favourable for AD, the low C/N ratio might have caused rapid acidification, resulting in lower efficiency. Li et al. [17] reported a biogas production value of 478.98 mL/g-VS at a 1:1 VS ratio of RS and pig manure. Contrary to this, Ye et al. [36] reported that a lower biogas production of 61.8 mL/g-VS was observed during the co-digestion of RS, pig manure, and kitchen waste as the mixture comprises a high content of kitchen waste.

The average methane yield per unit mass of volatile solids for all mixtures is presented in Figure 2. The ternary mixtures comprising FW and SS are likely to generate less methane than other substrates. This could be correlated with their degradation rates. FW and CM degrade easily, while SS and ChM degrade slowly. Hydrolysis occurs quickly in easily degradable feed, while methanogenesis is the rate-limiting step; on the other hand, among slowly degradable feeds, hydrolysis is slower. [42]. Ye et al. [36] reported a lower methane yield ranging from 13.33-60.20% for co-digestion mixtures of RS with kitchen waste and pig manure.

The graphs show that the A mixture produces the least biogas, the average methane content is below 40%, and digestion ends within 25 days. There is also no substantial difference in biogas value across the ranges of TS. In the case of mixture B, at TS 20%, the biogas production is 401.6 mL/g-VS, and from the 12th day, the productivity started increasing. The initial delay in biogas production might be due to the slow rate of degradability of the substrates. In combination D, digestion lasted 60 days, and biogas production is approximately 220 mL/g-VS for TS 15% and 20% and approximately 170 mL/g-VS for TS 25% and 30%. The lower production could be due to high total solids. Among all the combinations, the mixture of slowly degradable feedstocks has given stable results, i.e., mixture E. The results confirmed that the anaerobic co-digestion of two or more substrates could improve system stability and increase biogas production [43].

3.3 Total Volatile Solids Reduction

The rates of volatile solids removal for all the ternary mixes performed in AD is presented in Figure 3. The decrease in volatile solids content is an important parameter in assessing the performance of an anaerobic digester [19]. The combinations, A, B, and C showed around 50 % VS removal efficiency, whereas other ternary mixtures like E and F attained only 20-30% volatile solids reduction. The biodegradability of substrates improves as VS removal rates increase, and the initial high VS content of substrates allows better degradation. Despite low biogas and methane production values in mixtures containing FW, volatile solids removal efficiency ranges from 48.56 to 51.76%. There could be a difference in degradation efficiency due to the organic matter in co-substrates being more easily degradable than in RS. The organic contents present in FW are more easily degradable compared to other substrates. Ye et al. [36] reported a similar range of VS reduction (51.53-55.76%) during co-digestion of FW. In mixtures D, E, and F, however, the biogas productivity is good; comparatively, the VS reduction % is less may be due to the presence of SS and ChM.

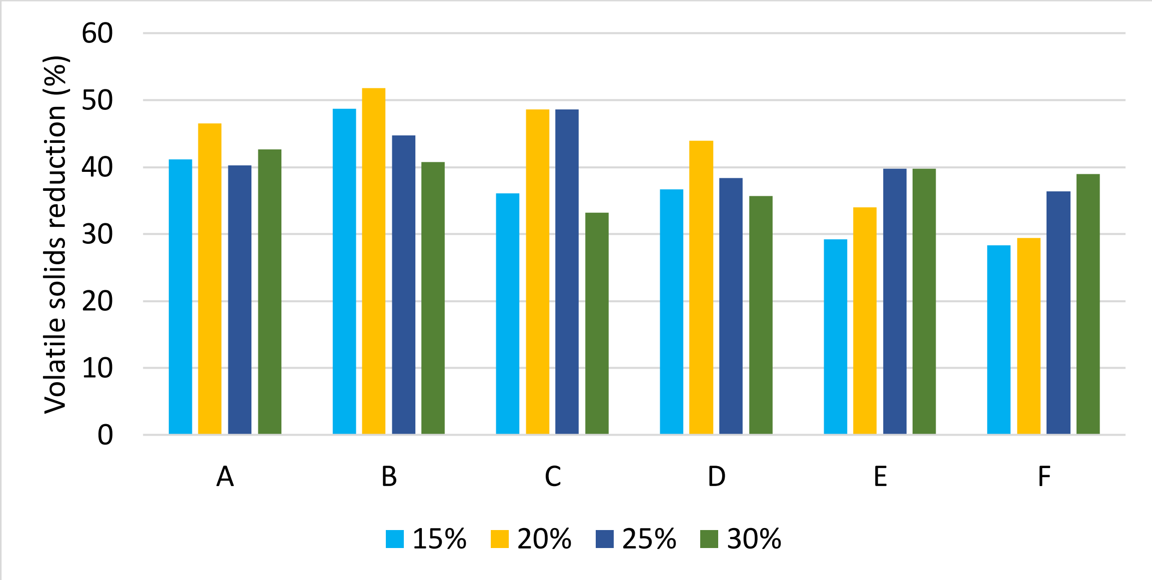


Figure 3: Volatile solids % in each mixture at all Total solid contents

3.4 Volatile Fatty Acids

Lignocellulosic matter degrades anaerobically primarily through hydrolysis (the rate-limiting step in quickly degrading feed), acidogenesis, acetogenesis, and methanogenesis (the rate-limiting step in slowly degrading feed). The efficiency of the hydrolysis and acidogenesis steps can be evaluated by the accumulated concentration of VFAs. The amount of VFAs is the indicator of acids produced from the hydrolysis and acidification process, which methanogenic bacteria cannot consume, on accumulation leads to a reduction of pH and system destabilisation [44]. Despite this, the indicative VFA level could not be specified with absolute certainty as the composition of the substrates and the operating conditions varied [45]. The accumulation of VFA restricts methanogenic bacteria, which disturbs AD by significantly lowering the pH levels [46]. VFA concentration of approximately 4000 mg/L inhibits the process [47]. The accumulation occurs due to the two-stage fermentation of the organic matter during the AD process. The acids generated during the hydrolysis and acidogenesis get converted into methane and carbon dioxide by methanogens in the methanogenesis [48].

The VFA variations of all the mixtures at all TS ranges are depicted in figure 3. Among the six ternary mixtures, A resulted in maximum VFA production of around 11448 mg/L at 20% TS which is far greater than the threshold limit (4000 mg/L). This could be correlated to the low range of biogas produced from the same mix. High VFA accumulation can also be related to the low C/N ratio of the substrates like FW. With a high VFA concentration, improved hydrolysis rates and degradation of RS recalcitrant lignocellulosic structure are observed, thereby enhancing biochemical conditions in the reactor and increasing biodegradability. A similar kind of inhibition was observed in mixtures C (20% TS) and B (25% and 30% TS) when the VFA generated crossed 10000 mg/L. The corresponding biogas generation values obtained at respective TS ranges are also lower (Figure 1) whereas, in the other three combinations i.e., D, E, and F, it is under the threshold value.

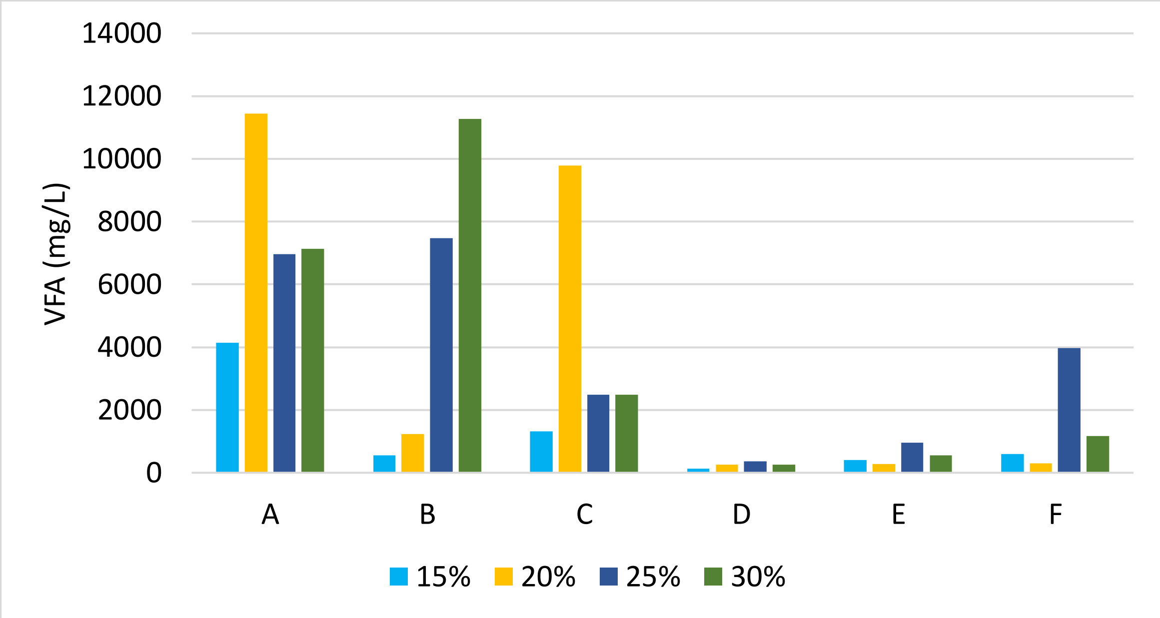


Figure 4: Volatile Fatty Acids (VFA) concentration in each mixture at all Total solid contents.

3.5 Kinetic Model Results on Ternary AD Mixtures

A better understanding of fermentation system evolution can be gained by analysing the kinetic parameters of the AD process. The cumulative biogas production values of each ternary mix at different TS% are simulated using a modified Gompertz model. The kinetic parameters estimated (P_{max}, Q, and ƛ) are summarised in Table 4, and the curves of model fitting are shown in Figure 6. The correlation coefficient (R^2) values obtained range from 0.90 - 0.99 for all mixtures at four TS contents showing that experimental values can be well simulated using the model chosen. AD efficiency can generally be determined by the maximum cumulative methane production potential (P_{max}) and the maximum methane production rate (Q). The maximum cumulative biogas production value obtained for mixture E at 20% TS was 470.01 mL/g-VS, and F at 20% TS with 447.47 mL/g-VS. Hence, the BMP values fitted well with the Modified Gompertz model. Wide variations in the lag phase time ($ƛ_{days}$) were observed. The lowest lag phase time was reported for mixtures comprising FW ranging from 0.03 to 4.46 days. This indicates that FW took a minimum of days to achieve the maximum biogas production. Furthermore, it was found that the anaerobic co-digestion of RS with CM and ChM has an apparent lag phase time ranging from 0.61 to 1.15 days. This value matches the results of Zhong et al. [49], where a lag phase time of 1.79 and 2.43 days was reported for RS and pig manure.

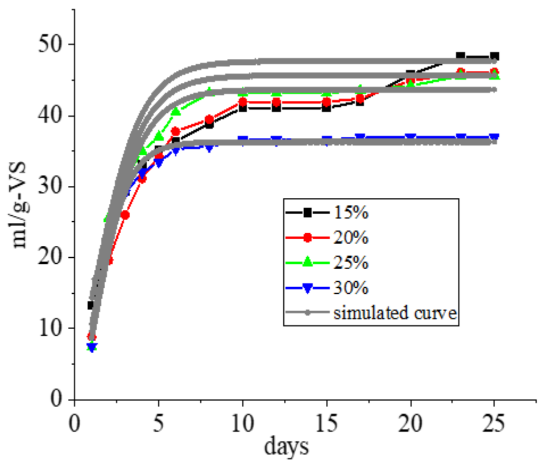
The highest lag phase time of 10.04 days was obtained for D at 30% TS. In A (all TS ranges), C (25%, 30% TS), E (all TS ranges), and F (all TS ranges), comparatively less lag phase times (0.03 to 4.46 days) are reported, which shows that methanogenesis can be accomplished in less time (Table 3). Other higher values of lag phase duration may be due to the mixing of easily degradable substrates for which methanogenesis serves as the rate-limiting step. Another reason could be the presence of high solids content. Gompertz's model predicted biogas yields between 0.33% and 15.65% higher than observed yields. Table 4. Shows the ANOVA test results of co-digestion experiment on each mixture. In an ANOVA test if *F – value* > *F – critical value*, then the results are significant or vice versa and *p – value* < 0.05 implies the results are significant or vice versa. From the Table 4, we can observe that *F – value* is greater than *F – critical value*, and *p – value* is less than 0.05 in all the co-digestion mixtures which depicts that the results of co-digestion are significant. In light of these results, it can be concluded that a study of the relationship between kinetic value and operational and process conditions can provide valuable insight into monitoring and controlling anaerobic co-digestion.

Table 3. Kinetic parameter values from Gompertz model analysis on co-digestion studies at four TS% ranges

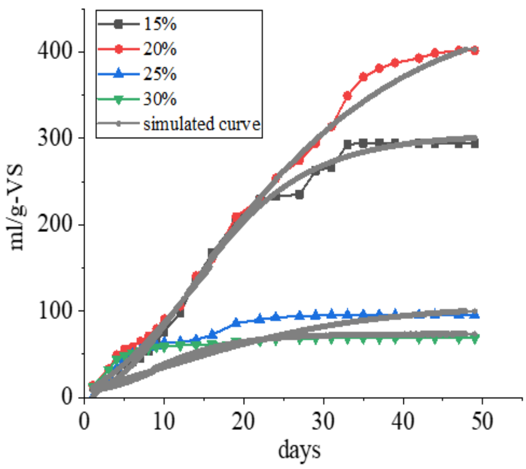
AD mixture Parameter	**Total solids %**			
A (RS+FW+CM)	15%	20%	25%	30%
P_{max}	47.49	45.44	43.67	36.29
$P_{experimental}$	48.33	46.10	45.61	36.91
Q	11.48	10.15	10.89	12.60
ƛ	0.52	0.81	0.03	0.32

R^2	0.90	0.92	0.96	0.98
Error (%)	1.73	1.43	4.25	1.67
B (RS+FW+ChM)				
P_{max}	304.26	402.95	99.88	73.31
$P_{experimental}$	294.34	401.61	95.48	68.55
Q	13.16	15.02	3.18	2.93
λ	3.73	0.47	1.68	2.15
R^2	0.99	0.99	0.95	0.96
Error (%)	3.37	0.33	4.60	6.94
C (RS+FW+SS)				
P_{max}	300.80	66.11	91.06	56.07
$P_{experimental}$	292.36	67.75	96.35	59.28
Q	9.57	5.44	7.95	5.52
λ	4.46	2.46	0.43	0.71
R^2	0.99	0.98	0.95	0.95
Error (%)	2.88	2.42	5.49	5.41
D (RS+SS+CM)				
P_{max}	248.19	235.23	193.56	198.55
$P_{experimental}$	245.40	212.09	169.31	171.67
R^2	0.99	0.98	0.94	0.93
λ	1.28	6.05	8.21	10.04
Q	6.67	5.46	11.91	4.41
Error (%)	1.14	10.9	14.32	15.65
E (RS+SS+ChM)				
P_{max}	362.87	470.01	324.79	299.06
$P_{experimental}$	357.31	407.60	315.16	277.91
Q	10.56	12.32	11.12	9.77
λ	0.91	1.33	1.45	1.98
R^2	0.91	0.98	0.99	0.99
Error (%)	1.55	15.31	3.05	7.61
F (RS+CM+ChM)				
P_{max}	299.10	447.47	221.75	94.00
$P_{experimental}$	295.95	441.99	216.00	92.79
Q	8.87	11.64	6.12	3.53
λ	0.94	0.61	1.15	0.86
R^2	0.98	0.99	0.97	0.97
Error (%)	1.06	1.23	2.66	1.30

a



b



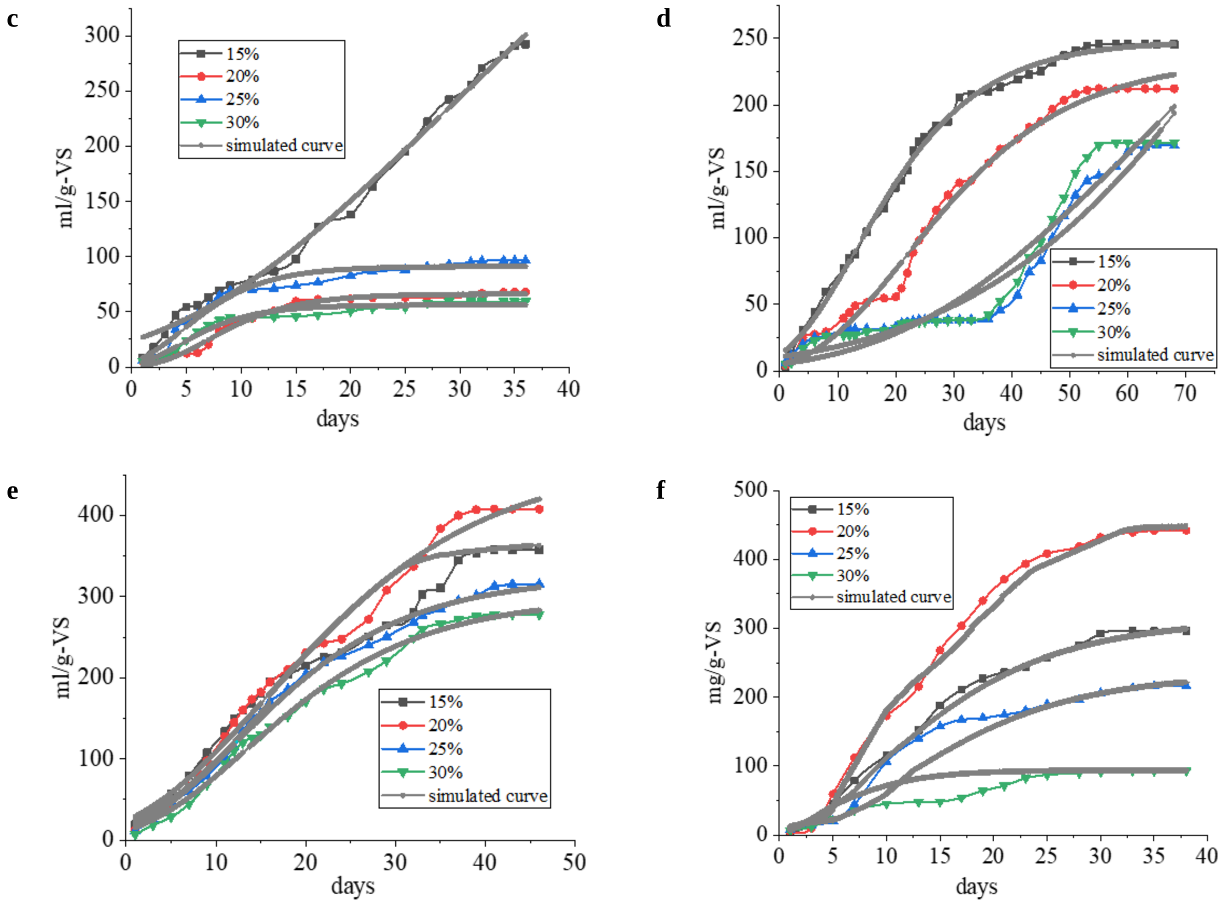
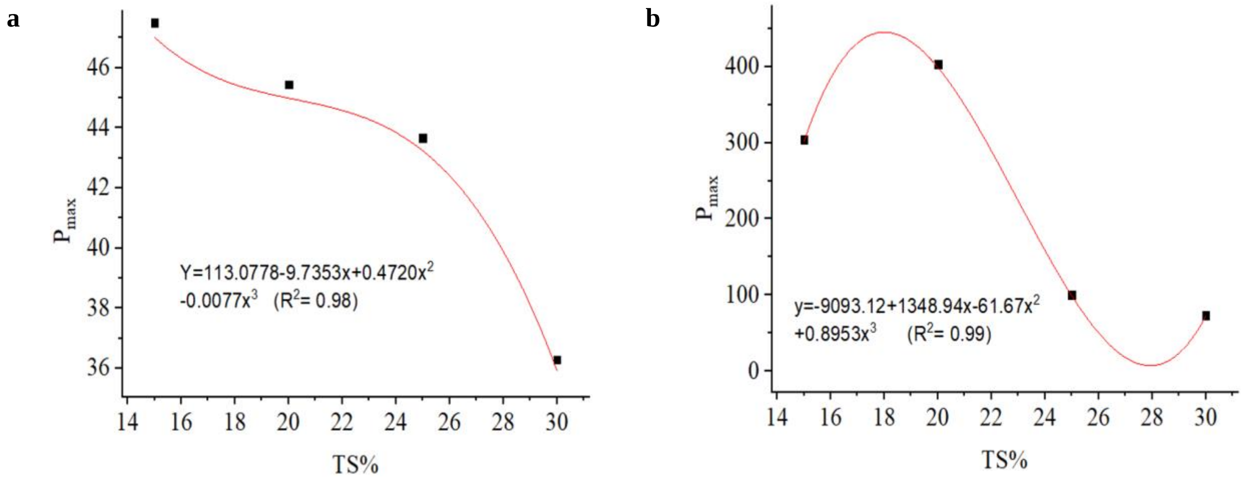


Figure 5: Graphs showing cumulative biogas yield plotted using the Gompertz model in mixtures A, B, C, D, E, and F respectively.



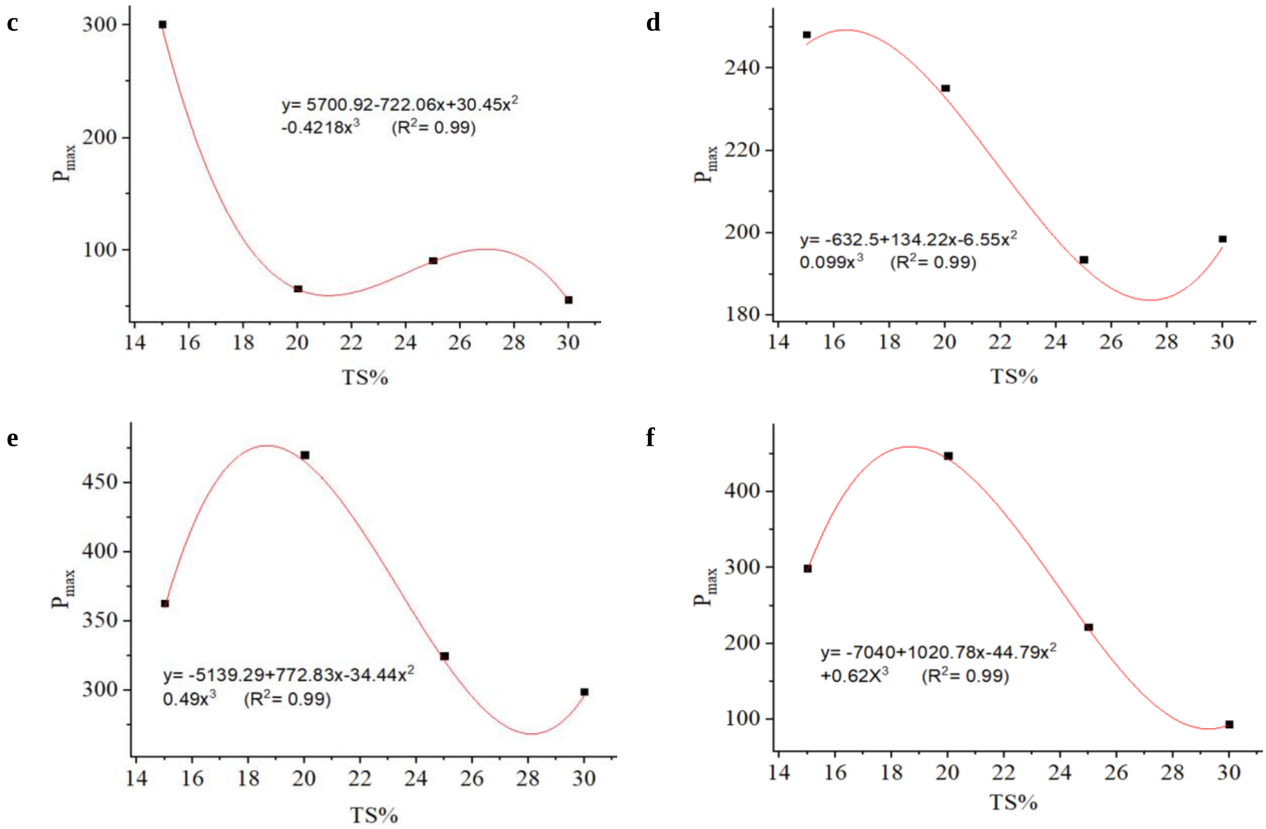


Figure 6: graphs showing maximum cumulative biogas yield plotted against TS% using the Gompertz model in mixture A, B, C, D, E, and F respectively showing the fit equation with R^2 value.

Table 4. ANOVA test results of co-digestion study

Co-digestion mixture	*F - value*	*p - value*	*F - critical value*
A (RS+FW+CM)	19.44	1.46E-10	2.51
B (RS+FW+ChM)	25.88	1.23E-15	2.44
C (RS+FW+SS)	21.30	4.98E-13	2.45
D (RS+SS+CM)	27.45	1.38E-17	2.42
E (RS+SS+ChM)	19.03	3.83E-12	2.45
F (RS+CM+ChM)	22.16	2.44E-12	2.48

4. Conclusion

The ternary mix combinations employed in this study using RS as the main substrate and FW, SS, CM, and ChM as co-substrates showed promising results in biogas production and degradability. At 20% TS, RS co-digested with ChM and CM, ChM and SS, and CM and SS produced maximum biogas production of 442, 407, and 245 mL/g-VS, respectively. Hence it can be concluded that co-digestion of RS with a binary mixture of SS, CM, and ChM is a competent approach for improving the biogas. Among the ternary mixes tested, maximum biogas production and methane content was obtained for the F mixture at 20% TS. A decrease in biogas productivity was observed for all the mixtures as TS% was increased. In addition, biogas production for mixture E at all TS contents was stable with no VFA accumulation, with a maximum production of 408 mL/g-VS at 20% TS. The maximum VS reduction was observed for the B mixture at 20% TS. VFA accumulation is much higher in A at all TS contents, possibly due to easily degradable substrates. The order of adaptability for choosing a co-substrate for RS can be listed as ChM>CM>SS>FW. The study has concluded that co-digestion with ternary mixtures is a systematic approach for enhancing biogas production.

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