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Anaerobic co-digestion of dry fallen leaves, fruit/vegetable wastes and cow dung without an active inoculum – A biomethane potential study

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ABSTRACT

Anaerobic digestion of fruit and vegetable wastes (FVW) is a sustainable energy production technology with a low carbon footprint but lacks consistent, productive process performance. Hence, the anaerobic co-digestion (AcoD) of FVW with various lignocellulosic biomass has become necessary. This study investigated AcoD of dry fallen leaves (DFL) and cow dung (CD) with FVW. Twelve combinations were designed, varying the proportion of DFL and FVW, keeping the amount of CD constant at 6 % total solids and 37 °C. The maximum biogas yield of 809 ± 96 mL/g VS_{input} was achieved in the reactor with a DFL to FVW ratio of 100:0. In contrast, a maximum methane yield of 388 ± 131 mL/g VS_{input} was observed in a reactor with a DFL to FVW ratio of 40:60, also saving 2 % (%w/w) of extra water than the former. The proposed co-digestion strategy has the potential for full-scale applications with its positive attributes.

1. Introduction

Anaerobic digestion (AD) is a low-carbon technology for sustainable energy production in the form of biogas from any kind of organic waste biomass (Chaudhary et al., 2022). Despite the beneficial characteristics of having high volatile solids (VS), AD of fruit and vegetable wastes (FVW) has been challenging for long-term stable operation (D' Silva et al., 2021a). This is because the inconsistent generation rate, varying composition with regards to time and regions and high rate of biodegradability of FVW lead to volatile fatty acids (VFAs) accumulation, disrupting the process stability (Isha et al., 2021, 2020). Various ways have been investigated to tackle the challenges; one of them is the anaerobic co-digestion (AcoD) of lignocellulosic biomass with FVW. The AcoD is a suitable strategy for achieving maximal biogas output and process stability, ultimately improving substrate degradation and microbial activity (Vats et al., 2019). Thus, waste biomass such as agricultural and agro-industrial residues, municipal sewage, sewage sludge, livestock wastes etc., have been well established as suitable co-substrates for AcoD of FVW (Ambrose et al., 2020; Arhoun et al., 2019; Bres et al., 2018; Fonoll et al., 2015; Gao et al., 2020; Vats et al., 2019; Wang et al., 2018).

The AcoD of sugarcane bagasse with fruit and vegetable wastes at a ratio of 30:70 (wet basis) reportedly generated maximum biogas yields, i.e., 87 mL/g TS in batch assay test (Vats et al., 2019). Wang et al. (2018) investigated the improvement in biomethane production through AcoD of cow dung (CD), corn stover and FVW. The best result of 743 mL/g VS in methane yield was achieved at a CD, corn stover and FVW mixing ratio of 28:1:5. The moderate amount of FVW among the combination of substrates solubilized more quickly than CD and corn stover enhanced the hydrolysis rate, which helped microbes gradually utilize the CD substrate. The study also added that corn stover became slowly accessible for AD bioprocess, adding very marginal methane yield relative to CD and FVW, acting primarily as a pH buffering agent.

Using lignocellulosic biomass as co-substrate poses challenges due to

Abbreviations: AcoD, Anaerobic co-digestion; AD, Anaerobic digestion; CD, Cow dung; C/N ratio, Carbon-to-nitrogen ratio; CPI, Co-digestion performance index; DFL, Dry fallen leaves; EC, Electrical conductivity; FTIR, Fourier transform infrared spectroscopy; FVW, Fruit/vegetable wastes; TA, Total alkalinity; TDS, Total dissolved solids; TS, Total solids; VFAs, Volatile fatty acids; VS, Volatile solids; XRD, X-ray diffraction.

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its partial recalcitrant nature. Pretreatments such as physical, chemical, thermal, biological or combinations are required to break down the lignin, which is detrimental to the fate of efficient biogas output (Kumar et al., 2021). More importantly, the availability of the recommended co-substrates is constrained to specific regions or the suitability of the local environment for its farming (e.g., agricultural wastes, livestock wastes etc.). A co-substrate available abundantly without any temporal and spatial variations might be the solution for this constraint.

Leaf litter with high cellulosic and hemicellulosic content is generally neglected in AD for biogas production, irrespective of its regional abundance. Utilizing leaf litter for biogas production is that it is available in abundance regardless of region, which is vital as the AD process is a viable technology for all types of communities for self-resilient and self-sustainable energy production, be it rural, urban, or remotely constrained (Vijay et al., 2021). Leaf litter and related plant/tree biomass have been commonly used for pyrolysis and gasification (Baghel et al., 2021; Khan et al., 2021). It has also been recognized as a bulking agent for aerobic composting while treating wet wastes (D' Silva et al., 2021b). Recently, leaf litter and fallen leaves have been investigated for biohydrogen and biogas production through dark fermentation or AD (Muhammad and Chandra, 2021; Yang et al., 2019). However, the studies have been too particular about leaf litter utilization from a specific tree species (Neem, *Azadirachta indica*) or the biofuel produced (biohydrogen or biogas). There is a need to study the mixed leaf litter's influence during co-digestion with FVW to stabilize the pH and carbon-to-nitrogen (C/N) ratio for enhanced biomethane production. The optimal C/N ratio is instrumental in preventing ammonia release or VFAs accumulation during the AD process, which balances the operational pH (D' Silva et al., 2021a; Kumar et al., 2021).

On the other hand, a properly developed microbial consortium is required to initiate the AD process. Fresh active inoculum from existing biogas or sewage treatment plants is recommended to inoculate new biogas plants in urban areas of developed and developing countries (Angelidaki et al., 2009). In rural areas, the active inoculum is not usually accessible and may require the localized development of an effective inoculum. The CD is a common inoculation substrate used in AD reactors in regions predominantly dependent upon agricultural/farm livelihood (Paes et al., 2020). The CD inoculated biogas plant is kept idle for at least 1 to 2 months to develop methanogens (Isha et al., 2021). The indigenously available microflora proliferates inside the digester during this idle condition. The addition of substrate along with fresh CD might early startup the microbial activity than without substrate addition, minimizing the time required for microbial development. Reducing the inoculum development period could also reduce the time needed to initiate the substrate feeding, which is directly linked with the waste biomass management and utilization of the region, regardless of its urban or rural nature.

We, therefore, hypothesize that high hemicellulosic and cellulosic nature with high volatiles and richness in the carbon content of dry fallen leaves (DFL) may stabilize nitrogen or protein-rich substances during AD. Also, the early introduction of the substrates with the CD may shorten the development time required for the inoculum. Hence, this study investigates the biomethane potential of AcoD of DFL, FVW, and CD without any external active inoculum. The biodegradability and co-digestion performance of DFL with FVW and CD are emphasized, and future opportunities are explored.

2. Materials and methods

2.1. Collection of substrates and preparation

The FVW and DFL used in this study were provided by the waste collection services from various student hostels and faculty apartments at the Indian Institute of Technology (IIT) Delhi campus, New Delhi, India. The required amount of fresh CD was collected from the dairy farm near the campus and used for the study after manually removing solid/inert particles. The FVW and DFL were pulverized/grinded as received before using them for the study. The FVW substrate was composed of peels, and unused portions of different fruit and vegetable wastes were pulverized thoroughly using a pulverizer (Power: 3.73 kW). Meanwhile, DFL consisting of leaves from various tree species was grinded thoroughly using a grinding machine (Power: 2.00 kW). The list of FVW and DFL species used for the experimental study is given in the supplementary files (see the supplementary materials).

2.2. Biomethane potential batch test and experimental design

The biomethane potential study was conducted in batch assays at Biogas Production, Enrichment and Bottling Laboratory, IIT Delhi. Each batch reactor had a total volume of 20 L and a working volume of 14 L. The batch reactors were fed with substrates (FVW, DFL and CD) at different combinations in triplicates, as shown in Table 1. The amount of CD was kept constant in all the reactors. The TS content of each reactor was set to 6 % TS by adding appropriate combinations of FVW, DFL and CD. Following the filling of the reactors with respective combinations of substrates and required water, the bottles were tightly sealed with rubber stoppers possessing two vents connected to stop cocks. To initiate the AD process inside the reactors, each reactor was purged with nitrogen for 5 mins to provide anaerobic conditions by flushing out air/oxygen. After confirming that the reactors were well sealed, the reactors were placed inside a temperature-controlled environment maintained at a mesophilic temperature of 37 °C. After 24 h, one of the stop cocks in each reactor was opened to pass out the gas (predominantly nitrogen gas) inside the reactor. From then on, the experiment was considered initiated, and the next day was considered Day 0 of the study. The volume of the produced biogas was determined using the water displacement method every 1 to 5 days.

The biogas composition (CH_4, CO_2, H_2S and residual other gases in percentage) was analyzed using a calibrated Biogas Analyzer (Model: Biogas-5000, Geotech, UK). Each reactor was shaken manually before and after the biogas volume determination and composition analysis. The study was continued till the biogas production from the reactors ceased completely. The biogas volume was further converted to standard temperature and pressure of 0 °C and 1.01 bar.

2.3. Analytical methods

The substrates, and the samples from the reactors during the initial and final days were subjected to various analyses. The proximate analyses (moisture content (MC), TS, VS and non-volatile solids (NVS)) and total alkalinity (TA) of the samples were carried out according to the standard methods (APHA, AWWA,WEF, 2012). The ultimate analysis (carbon, C; hydrogen, H and nitrogen, N values in percent) was carried out using an automated 'Vario EL' elemental analyzer (Make: Perkin Elmer, USA). DFL and CD samples' compositional analysis (lignin, cellulose, and hemicellulose) was carried out as described in Datta (1981). The analysis of pH, total dissolved solids (TDS) and electrical conductivity (EC) of the initial and final day reactor samples were carried out using PCTestr 35 multiparameter test kit (Make: OAKTON, Eutech Instruments, U.S.). The DFL sample was subjected to X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy analyses. The samples were ground and oven-dried thoroughly and passed through a sieve with a pore diameter of 0.212 mm before the analyses. FTIR spectroscopy was carried out using NICOLET-IS-50 (Thermo Fisher Scientific, United States) using a KBr to sample ratio of 100:1 (Kumar et al., 2019), while XRD analysis using X'Pert PRO (PANalytical, Netherlands) at Central Instrumentation Facility, Indian Institute of Technology Delhi, New Delhi. The crystallinity of the fallen leaves' samples was calculated from the XRD peaks, as in Eq. (1):

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

Table 1
Reactor configuration and experimental design.

Reactor nomenclature	Reactor working volume (L)	DFL:FVW ratio	DFL added (g)	FVW added (g)	CD added (g)	Water added (L)	TS (%)	No. of replications
R1	14	–	–	–	1050	12.95	3	3
R2	14	100:0	230.77	0	1050	12.71	6	3
R3	14	90:10	227.16	25.24	1050	12.69	6	3
R4	14	80:20	222.81	55.70	1050	12.67	6	3
R5	14	70:30	217.46	93.20	1050	12.63	6	3
R6	14	60:40	210.70	140.47	1050	12.60	6	3
R7	14	50:50	201.92	201.92	1050	12.54	6	3
R8	14	40:60	190.05	285.07	1050	12.47	6	3
R9	14	30:70	173.08	403.85	1050	12.37	6	3
R10	14	20:80	146.85	587.41	1050	12.21	6	3
R11	14	10:90	100.96	908.65	1050	11.94	6	3
R12	14	0:100	0	1615.38	1050	11.33	6	3

where I_{002} is the intensity of diffraction from the 002 plane at $2\theta = 22°$ and I_{am} is the intensity of background measured at $2\theta = \sim 18.6$ °C.

2.4. Kinetic modelling, co-digestion performance index, theoretical biogas/biomethane potential and statistical analyses

The experimental biogas and methane yields were fitted with the modified Gompertz kinetic model, one of the most preferred models by previous literature (Isha et al., 2021). The parameters such as maximum biogas/biomethane production potential, daily biogas/biomethane production rate and lag phase were simulated from the model as reported in earlier literature (Isha et al., 2021) as shown in Eq. (2).

$$M = P_b \times exp\left\{ - exp\left[\frac{R_m.e}{P_b}\right](\lambda - t) + 1 \right\} \tag{2}$$

where M is the biogas yield (in mL/ g. VS) with respect to time t (in days), P_b is the maximum biogas/biomethane potential of the substrate (in mL/ g. VS), R_m is the maximum biogas/biomethane production rate (in mL/ g. VS), λ is the lag phase time taken for biogas/biomethane production (in days), e is the Euler's function which is equal to the value 2.7183 (Isha et al., 2021).

In AcoD, an increased biomethane yield is expected since the organic content of the co-digested substrates is higher than that of the mono-digestion. Additionally, combining more than one substrate might also have a synergistic effect. The synergistic effects may arise from the dilution of inhibitory/toxic by-products. The nutrients in the substrates complement each other, resulting in increased biodegradability and a shift in the microbial consortia with more improved metabolism (Hou et al., 2020). Thus, to evaluate the synergistic effect of substrates in the AcoD process, Labatut et al. (2011) recommended comparing the biomethane potential of the AcoD results with the weighted sum of the biomethane potential of the individual substrates as a measure of synergistic or antagonistic effects.

Further, an indicator known as the co-digestion performance index (CPI) was established to determine the performance of the co-digestion experiment (Ebner et al., 2016). The CPI is the ratio of the biomethane potential of the co-digested mixture (B_i) to the weighted average (B_{0i}) of the individual biomethane potential of the substrates ($B_{0,\,i}$) (Ebner et al., 2016):

$$CPI = \frac{B_i}{B_{oi}} = \frac{B_i}{\sum_i^n \%VSB_{o,i}} \tag{3}$$

The biodegradability index represents the performance of the AD process with respect to substrate degradation and biogas/biomethane production. The theoretical biogas (B_{th}) and biomethane potential (M_{th}) were calculated by considering the elemental analysis results as per Boyle's Law (Eqs. (4), (5) and (6)) (Nielfa et al., 2015). The biodegradability index was calculated as the ratio of experimental values (biogas/biomethane) obtained to the calculated theoretical values (biogas/biomethane) (Eq. (7)).

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3 \tag{4}$$

$$B_{th}\left(mL/_{g.VS}\right) = \frac{a22.415}{12a + b + 16c + 14d} \tag{5}$$

$$M_{th}\left(mL/_{g.VS}\right) = \frac{\left(\frac{4a+b-2c-3d}{8}\right)22.415}{12a + b + 16c + 14d} \tag{6}$$

$$Biodegradability\ index\ (\%) = \frac{Experimental\ biogas\ or\ biomethane\ potential}{B_{th} or M_{th}} \tag{7}$$

The statistical analyses carried out in this study were done using the Microsoft Office Excel 360 version with a confidence interval of 95 %, and model simulations were done using the solver function.

2.5. Energy balance

The reactors' energy balance was determined by calculating the net energy output. The energy output of the reactors was calculated considering the methane volume produced and was converted to heat and electrical energy with a 10 % loss. Meanwhile, the energy input required for each reactor was calculated separately, considering the power and time taken for grinding and pulverization of the DFL and FVW substrates. The equations used (Eqs. (8) and (9)) for the calculations are as in Isha et al. (2021).

$$Electricity\ generated\ from\ biogas\ produced\ (in\ kWh) = \frac{Biogas\ produced\ (m^3) \times LHV_{CH_4} \times Methane\ content\ (\%) \times Engine\ efficiency}{3.6MJ/kWh} \tag{8}$$

$$Heat\ generated\ from\ biogas\ produced\ (in\ MJ) = Biogas\ produced\ \left(m^3\right) \times LHV_{CH_4} \times Methane\ content\ (\%) \times Engine\ efficiency \tag{9}$$

$$Energy\ balance\ (\%) = \frac{Energy\ output - energy\ input}{Energy\ output} \times 100 \tag{10}$$

$$Energy\ ratio = \frac{Energy\ output}{Energy\ input} \tag{11}$$

For the calculations, it was assumed that methane's lower heating value was around 35.59 MJ/m^3, 40 % engine efficiency for electrical energy production and 50 % for heat energy production and an electricity conversion factor of 3.6 MJ/kWh. The energy balance was determined according to the calculated net energy output (MJ) divided by the energy output (MJ). At the same time, the energy ratio was calculated as the ratio of energy produced (MJ) to the energy input required (MJ). The energy ratio above value 1 is evaluated as highly effective with respect to energy recovery (Gaur et al., 2017).

3. Results and discussion

3.1. Characterization of substrates used

The possibility of using DFL as a suitable co-substrate was primarily validated through various analyses before the biomethane potential experiment. This includes the physical, proximate, ultimate and bromatological analyses, as shown in Table 2. The physical characteristics of the substrate, such as pH, water retention capacity, solubility, and swelling, are more related to the process reactions in AD and bulk density is associated with the designing of the digesters (Dumas et al., 2015).

The DFL substrate possesses a higher TS and VS content with low moisture content. Similarly, the ultimate analysis results revealed that the DFL contained more carbon than FVW and CD. In general, lignocellulosic biomass is bound to have high dry matter (TS and VS) content, as was the case for rice straw, wheat straw etc., as reported by various researchers (Chandra et al., 2012; Kumar et al., 2019). The nitrogen content of the DFL was reported as 1.88 ± 0.24 %, which almost exceeded the values of the FVW and CD that reported 1.43 ± 0.12 % and 1.50 ± 0.06 %, respectively. Thus, the C/N ratio of DFL, FVW, and CD was found to be 21.77 ± 0.95, 23.84 ± 1.18 and 21.85 ± 1.84, respectively. It shows that DFL can be anaerobically mono-digested as well, as the optimal C/N ratio for maximal biogas production is between 20 and 30 (Isha et al., 2021; Kumar et al., 2021).

It is noteworthy that being physically dry in nature, the disadvantage of mono-digestion of DFL might require a high amount of water for the dilution to maintain a fixed feeding rate in the digestion system. Hence, co-digestion of DFL with substrates containing high MC such as FVW and CD could be the best strategy for improved energy recovery and reduced resource requirements, which is to be investigated through this study.

According to the FTIR characterization of DFL, the broad peaks observed at different wavelength ranges confirms the presence of phenols, alcohols, alkyl methylene, monocyclic substituted aromatics, carbonyl, and hydroxyl groups. The presence of C═O stretching at 1500 to 1800 cm^{-1} also indicates the hydrophilic nature of the substrate (Nieto-Gligorovski et al., 2008), which can play a crucial role in the solubilization and early hydrolysis. These chemical functional groups have been reported to be very suitable for AD and possess essential nutrients for microbial growth (Khayum et al., 2018).

The DFL contained hemicellulose content of 20.95 ± 0.82 %, cellulose content of 9.17 ± 0.48 %, the lignin content of 4.97 ± 0.37 % (see Table 2). The FTIR also confirmed the presence of hemicellulose and cellulose contents higher than lignin content with functional groups of —CH_2, C—H, C—H_2, and COC stretching, C═O stretching of acetyl or carboxylic acid, and C—O stretching between the wavelength of 1050–1450 cm^{-1}. The lower lignin content and higher amount of extractives and soluble matter in the DFL substrate benefit AD. Lower the lignin content, the pretreatment required for the lignocellulosic breakdown of the substrate might not be required (here in the case of DFL). Hence, no pretreatment was carried out for DFL other than the usual size reduction through grinding, i.e., generally recommended (Fernandez et al., 2022; Kumar et al., 2021).

Cellulose crystallinity is a parameter that reflects the proportion of the crystalline region of cellulose. The crystallinity index of lignocellulosic biomass determines its biodegradability during AD. A sharp peak at 2θ values between 18 and 22° owes to the presence of crystalline cellulose in the biomass samples (Awoyale and Lokhat, 2021). The crystallinity index of DFL was much lower than the values of other common lignocellulosic biomass (see Table 3), with a value of 20.64 %. It could be due to the difference in the composition of the DFL substrate as compared to commonly known lignocellulosic biomass, as explained earlier. The non-existence of sharp peaks proves the amorphous texture of the DFL biomass (Awoyale and Lokhat, 2021). The higher presence of hemicellulose, soluble matter, and amorphous cellulose over crystalline cellulose in the substrate might have overturned the crystallinity index to a lower value. When anaerobically digested, readily available extractives and hemicelluloses might improve the degradation rate. This is another reason why no other pretreatment technique was not recommended for this study. The FTIR and XRD characterization plots are in the supplementary file (see the supplementary materials).

Table 2
Properties of dry fallen leaves with other recommended co-substrates in literature.

Parameters	Unit	DFL	FVW	CD
Physical analysis				
pH	–	7.23 ± 0.01	5.63 ± 0.05	7.67 ± 0.26
Water retention capacity	g_{water}/g_{DM}	4.20 ± 0.14	–	–
Solubility	$g_{solubles}/g_{DM}$	3.00 ± 0.14	–	–
Swelling	mL_{water}/g	3.10 ± 0.14	–	–
Bulk density	kg/m^3	285.00 ± 1.41	–	–
Proximate analysis				
Moisture content	%	10.36 ± 0.04	85.35 ± 1.26	76.72 ± 1.29
Total solids	%	89.64 ± 0.04	14.65 ± 1.25	23.28 ± 1.29
Volatile solids	%	66.99 ± 0.16	13.15 ± 0.60	18.46 ± 1.63
Non-volatiles	%	19.77 ± 0.25	10.22 ± 0.60	4.82 ± 1.63
Ultimate analysis				
Carbon	%	40.90 ± 3.40	33.85 ± 0.24	32.55 ± 1.45
Hydrogen	%	5.46 ± 0.14	5.37 ± 0.30	4.93 ± 0.27
Nitrogen	%	1.88 ± 0.24	1.43 ± 0.12	1.50 ± 0.06
C/N ratio	–	21.77 ± 0.95	23.84 ± 1.18	21.85 ± 1.84
Compositional analysis				
Hemicellulose	%	20.95 ± 0.82	–	15.86 ± 0.44
Cellulose	%	9.17 ± 0.48	–	19.92 ± 0.93
Lignin	%	4.97 ± 0.37	–	16.31 ± 1.12
Lignocellulose	%	35.09 ± 0.03	–	52.09 ± 0.63
Solubles	%	64.91 ± 0.03	–	47.91 ± 0.63
Bromatological analysis				
Crude fibre	%	2.99 ± 0.22	–	–
Crude proteins	%	0.12 ± 0.01	–	–
Carbohydrates	%	–	–	–

Table 3
Comparison of crystallinity index with other recommended co-substrates for FVW-AD.

Lignocellulosic biomass	Crystallinity index (%)	Reference
Wheat straw	45.70	Kumar et al., 2019
Pearl millet straw	44.50	Kumar et al., 2019
Pine wood	35.73	Darmawan et al., 2016
Rice straw	52.20	Singh et al., 2014
Dry fallen leaves	20.64	This study

3.2. Evaluating the performance of biomethane potential study

3.2.1. Biogas, biomethane production and kinetics

The biogas production was initiated in all reactors within the first week. During week 2, high weekly biogas production of 140 ± 70 mL/g VS_{input}, 34 ± 17 mL/g VS_{input} and 59 ± 29 mL/g VS_{input} were exhibited by reactors R2, R3 and R4, respectively. However, during week 3, the highest weekly biogas production was observed in reactors R1, R5 to R8 and R10. The reactors R9 and R12 provided significant biogas yields above 30 ± 8 mL/g VS_{input} only by week 4, and in reactor R11, it required even more time. The maximum cumulative biogas production was observed for the reactor R2 reactor (significant using one-way ANOVA, $p < 0.05$, $f > f_{crit}$), followed by R8. The cumulative biogas production for the R2 reactor was 809 ± 96 mL/g VS_{input} and 799 ± 157 mL/g VS_{input} for the reactor R8, respectively. The results were almost similar to the biogas production yield obtained during mesophilic batch AD of Chinese cabbage residue and Chinese cabbage mixture reported by Tang et al. (2020), i.e., 800 ± 62 mL/g VS and 787 ± 100 mL/g VS, respectively, at a temperature of 35 °C and TS content of 4 %. The study has suggested that a highly active microbial community was involved in the AD process.

Biogas production from reactor R1 (CD alone) took 40 days to produce 90 % (T_{90}) of its total biogas produced (237 ± 155 mL/g VS_{input}), which is the recommended hydraulic retention time (HRT) for CD substrate (Isha et al., 2021; Jha et al., 2021). The experimental values of all the reactors were simulated with the modified Gompertz model that provided the values of $P_{bbiogas}$, $R_{mbiogas}$, and λ_{biogas}. The $P_{bbiogas}$ and $R_{mbiogas}$ values were highest in reactor R2, predicted with a minimal error (R^2: 0.99, Error: 2.81 %) by the modified Gompertz model. This was followed by reactor R8 with the $P_{bbiogas}$ and $R_{mbiogas}$ values of 808 mL/g VS_{input} and 16 mL/g VS_{input}.d. From Table 6, it can be seen that the higher proportion of DFL than the FVW proportion resulted in much lower λ_{biogas} (<9 d), indicating the early hydrolysis and AD process startup (Isha et al., 2021). It was also concluded from the weekly biogas productions, as discussed earlier.

The high soluble matter present in the DFL and its neutral nature might have aided in the early startup of the process. However, out of the total digestion period taken for the study, i.e., 140 d, the DFL and FVW took 100 d for complete digestion, excluding the time taken by CD substrate only (reactor R1 results). This discrepancy could be due to the non-availability of active microbes at the initial days of the experiments. Methanogens are known for their slower growth rate (Gerardi, 2003). Carbon and nitrogen are the primary nutrients with other trace elements for the synergistic growth of microbes inside the AD reactor (Khayum et al., 2018).

Another aspect to be taken into account for increased biodegradability is the type of inoculum used, its collection source and the microbes involved. Hence, the substrate to inoculum ratio and stabilization of process parameters play a significant role in the biodegradability of organic waste fractions during AD (Yoon et al., 2014). As a result, the biodegradability index with respect to biogas production was much higher for the reactors R2 and R8 achieving up to 58.95 % and 61.78 %, respectively. The reactors R4, R9, R11 and R12 also achieved a biodegradability index above 50 %, attaining the values of 51.87 %, 52.45 %, 59.24 %, and 58.27 %, respectively. Thus, the experimental values were much lower than the theoretical estimations, which is expected in the case of AD. This is because the carbon and other nutrients are not only used as a food source but are also assimilated in the cells of anaerobic microbes (Lesteur et al., 2010; Nielfa et al., 2015). The weekly and cumulative biogas profiles and modified Gompertz model simulations are in the supplementary file see the supplementary materials).

Although initiation of biogas production is essential, its composition must be emphasized, especially with the methane content that possesses the energy (Isha et al., 2021).

Fig. 1 represents the weekly and cumulative methane yield obtained in all the reactors during the complete experimental run. When the biogas production yield in the reactors initiated from the 1st week itself, significant methane yields were observed only from the 2nd week. The reactor R2 recorded a weekly methane yield of 41 ± 21 mL/g VS during the 2nd week. During the succeeding week (3rd week), the reactors R1,

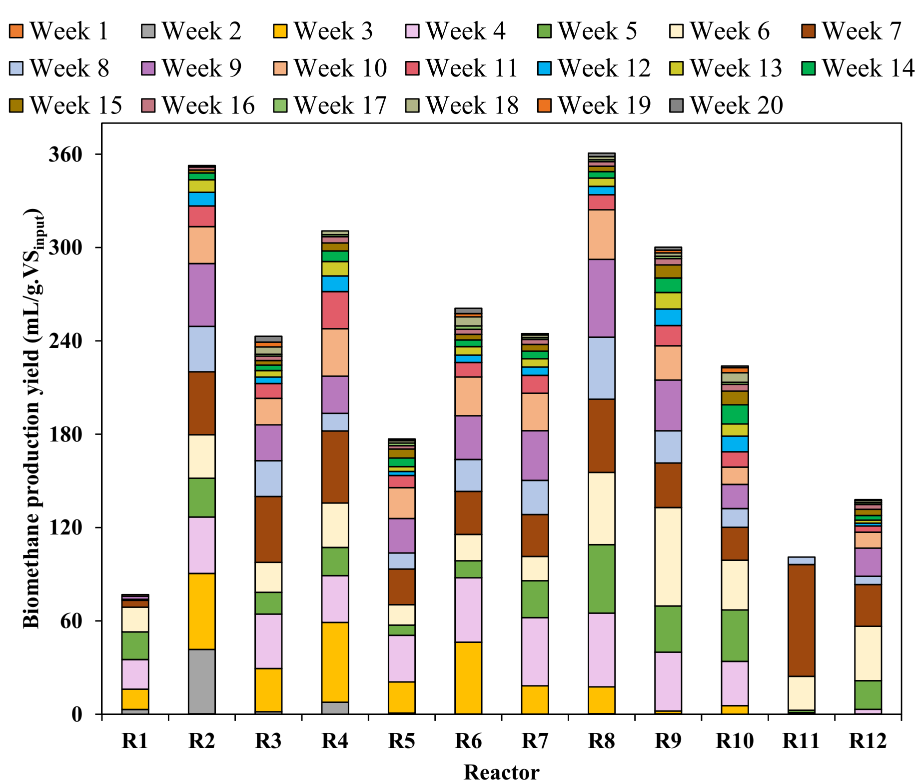

Fig. 1. Weekly and cumulative biomethane production profile in all the reactors over the experimental run.

R3 to R8 recorded significant methane yields ranging from around 13 ± 6 mL/g VS_{input} to 51 ± 16 mL/g VS_{input} per week. The methane production stabilized or increased in all reactors until the 12th week of the experimental study.

A maximum methane yield of 388 ± 131 mL/g VS_{input} and 353 ± 38 mL/g VS_{input} were obtained in R8 (confirmed significant using one-way ANOVA, $p < 0.05$, $f > f_{crit}$) and R2 reactors, respectively. The biomethane yield from reactor R8 was 9.17 % higher than reactor R2 and 19.99 % higher than reactor R4 (311 ± 15 mL/g VS_{input}). This contradicted the experimental results for biogas production, where reactor R2 biogas yield exceeded all the reactors. The DFL addition might have propagated early hydrolysis, but when it comes to methane production, the increased amount of FVW aided in the methanogenic activity due to higher solubilization. This might be the reason for the discrepancy between biogas production and biomethane production results. The reactors with a lower (R3 to R7) and higher (R9 to R12) proportion of FVW recorded lower methane yield. The fluctuations in VFA production, lower solubilized material availability and reduced pH could be the reason behind lower methane yield.

The experimental methane yield obtained in reactor 8 is almost indistinguishable from the reported values during AcoD of food wastes and heterogenous straws (maize, sorgos, and wheat). The study reported a maximum methane yield of 392 mL/g VS at food wastes to the straw mixing ratio of 5:1 at 35 °C (Yong et al., 2015) and higher than the values of 289 mL/g VS was reported during AcoD of tomato plant waste and corn stover at a ratio of 7:3 on VS and C/N ratio basis (Szilágyi et al., 2021). The studies cited that properly utilizing the released VFAs during the co-digestion resulted in increased methane yield under mesophilic conditions. However, these experimental results are lower than the reported values of 430 ± 28 mL/g VS_{input} during co-digestion of food waste and cattle dung with the mixing ratio of 60:40 at an operating temperature of 37 °C (Zamanzadeh et al., 2017). The variation in reactor configuration and substrate composition might have led to varied methane yield in the present study.

Table 4 represents the comparison of experimental biomethane yield with the simulated values. In reactor R8, even though the biogas production was initiated with a λ_{biogas} value of 15.01 and $\lambda_{biomethane}$ value of 17.64, the stabilized process parameters might have provided a suitable environment for the methanogenic bacteria to culture, thrive and actively participate in an AD process (D' Silva et al., 2021a; Isha et al., 2021).

It was evident that methane production in the reactors took more time since the methanogens require more days for proper growth and development. Hence, the values of $\lambda_{methane}$ from each reactor were compared with the obtained λ_{biogas}. It clarifies the time taken for the methanogenic activity. The lowest time taken to record the methanogenic activity was found in the reactor R8 with a value of 2.63, which is much lower than the expected growth rate reported for methanogens, i.e., 4 days (Gerardi, 2003). The reactors R1, R5, R6, R10 and R12 recorded $\lambda_{methane}$ values around 4.13 to 4.78. The $\lambda_{methane}$ values of the remaining reactors were found to be above 5.53, reaching up to 11.67.

The biodegradability index with respect to the experimental methane yield was also calculated compared with the theoretical methane yield. The biodegradability index of reactor R1 was about 34.71 % with respect to the methane yield, which is 11.13 % less than the biodegradability index obtained for biogas yield. Similarly, the reactors R5, R10, R11 and R12 that reported lower methane yield compared to biogas yield have directly affected the difference between biodegradability indices. However, the reactors R2, R3, R4, R6, R7, R8, and R9 reported higher biodegradability with respect to methane yield over the biogas yield. This confirms that the methanogens were more active and provided stable methane production in these reactors.

3.2.2. Co-digestion performance index and synergistic effect assessment

Fig. 2 shows the CPI of the reactors R1 to R12. In this study, the reactors R1 to R12 exhibited CPI values of 0.20, 0.95, 0.65, 0.83, 0.47, 0.70, 0.66, 1.04, 0.81, 0.60, 0.27, and 0.26, respectively. The CPI values are classified into two classifications as synergistic and antagonistic keeping the baseline as the value 1 (Hou et al., 2020). If the CPI is >1, the CPI performance is considered synergistic, while when the value is <1, then the performance is antagonistic (Hou et al., 2020). Among the reactors operated in this experimental study, only the reactor R8 showed a synergistic effect, suggesting that the mixing ratio (DFL: FVW) of 40:60 is the only ratio that positively impacts co-digestion over mono-digestion of these substrates.

A similar CPI value of 1.03 was reported during co-digestion of food waste and rice straw at a ratio of 50: 50 (Hou et al., 2020) and during the co-digestion of food waste, human feces, and toilet paper regardless of the mixing ratio (Kim et al., 2019). A CPI value of 1.02 was reported during manure and various crop residues at a ratio of 50:50 and a value of 1.05 during AcoD of crop residues and municipal solid wastes at a mixing ratio of 50:50 (Pagés-Díaz et al., 2014).

All these values are lower considering the CPI value of 1.24 reported in a former literature on co-digesting food waste and rice straw (Hou et al., 2020) and 1.29 during co-digestion of slaughterhouse waste, various crop residues and manure at a mixing ratio of 33:33:33 (Pagés-Díaz et al., 2014). The experimental results in this study suggest that the DFL substrate proportion has a positive impact since the reactors R2, R4 and R8 showed CPI values above 0.83, the value reported during the co-digestion of food waste and dairy manure (Ebner et al., 2016). This means DFL does have a synergistic effect on AcoD with FVW and CD, according to the present study.

The observations made in this study imply that the co-digestion of FVW with DFL and CD can provide synergy-producing energy. The fundamental mechanism behind this synergistic effect could be attributed to the mixtures' characteristics and proportions, nutrient balance,

Table 4
Experimental results, kinetic parameters, theoretical estimations, biodegradability, and co-digestion performance of the reactors for biomethane production.

Reactor name	Experimental biomethane produced (mL/g. VS_{input})	Predicted biomethane produced (mL/g. VS_{input})	$P_{b\_methane}$ (mL/g. VS_{input})	$R_{m\_methane}$ (mL/g. VS_{input}.d)	$\lambda_{methane}$ (1/d)	T_{90} (d)	T_{eff} (d)	R^2	Error (%)	Theoretical biomethane production (mL/ g. VS_{input})	Biodegradability index (%)
R1	76 ± 31	76.40	76.40	3.27	15.27	41	25	0.99	0.32	219.42	34.71
R2	353 ± 38	362.03	365.07	5.84	6.99	70	63	0.99	2.55	561.82	62.78
R3	243 ± 61	239.20	240.73	4.27	14.51	85	70	0.99	1.57	552.59	43.97
R4	311 ± 16	317.35	322.15	4.75	10.13	80	69	0.99	2.08	543.37	57.17
R5	177 ± 67	179.03	181.52	2.78	13.15	89	75	0.99	1.13	534.15	33.13
R6	261 ± 63	258.67	261.71	4.03	9.65	87	77	0.99	0.87	524.92	49.71
R7	245 ± 123	247.68	249.59	4.35	16.02	90	74	0.99	1.22	515.70	47.44
R8	388 ± 131	390.45	391.41	8.25	17.64	67	49	0.99	0.54	506.47	76.67
R9	300 ± 29	294.96	296.34	5.79	20.16	88	67	0.99	1.78	497.25	60.38
R10	224 ± 21	215.87	218.45	3.52	15.88	97	81	0.99	3.66	488.02	45.87
R11	101 ± 52	101.66	101.66	10.65	38.64	48	9	0.99	0.62	478.80	21.10
R12	96 ± 57	92.63	92.70	2.46	24.88	88	63	0.99	4.03	469.57	20.52

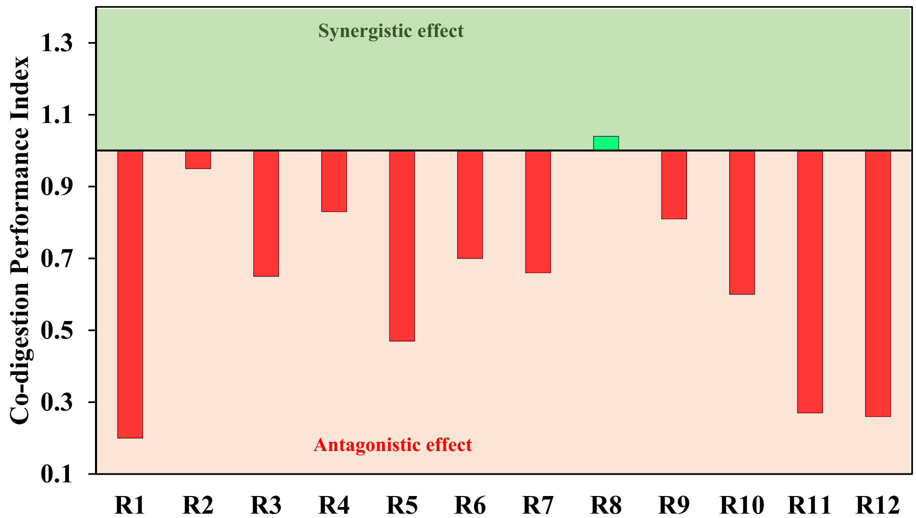

Fig. 2. Co-digestion performance index and synergistic/antagonistic effect of the reactors.

stimulated microbial synergism, increased buffering capacity, and dilution of the toxic compounds during the digestion process (Hou et al., 2020; Kim et al., 2019). Further to validate the results, process parameters of pH, TDC, EC and TA were analyzed for the initial and final samples and discussed in Section 3.2.3.

3.2.3. *Variations in process parameters and mass balance*

Table 5 shows the variation in process parameters during the initial and final phases in all the reactors. The results show that there are significant changes in all measured physico-chemical process parameters during the AcoD process. The pH plays a vital role in the AD process from the initial hydrolytic stage to the methanogenic stage (D' Silva et al., 2021a; Isha et al., 2021). It can be observed from the initial pH values of the feeding mixture reduced as the amount of DFL reduced from 6.76 ± 0.22 in the reactor R1, 6.55 ± 0.01 in the reactor R2 to 4.07 ± 0.03 in the reactor R12. As discussed in the earlier Sections 3.2.1 and 3.2.2, λ_{biogas} and $\lambda_{methane}$ increased as the proportion of FVW substrate increased, which could be because the reactors' pH was not sufficient for the early hydrolysis, which is generally recommended near to 6.0 (D' Silva et al., 2021a).

On the other hand, the TDS and EC values increased as the FVW increased, indicating its high biodegradable characteristics. The TDS values in the reactors R1 to R6 were ranged from 1500 ± 212 to 1990 ± 184 mg/L; meanwhile, this increased from 1840 ± 99 to 3380 ± 665 mg/L for the next 6 reactors (R7 to R12). The TDS concentration increases in the effluent as the organics are solubilized than the TDS concentration recorded at the inlet. The EC also varied along with the TDS concentration. Similar observations were previously reported in an anaerobic digester treating food and dairy wastes by Pannase et al. (2020). It could be because TDS and EC are directly correlated with each other (D' Silva et al., 2021b). EC values in the reactors R1 to R6 were determined between 2110 ± 269 and 2760 ± 283 μS/cm, which was further increased up to 4775 ± 940 μS/cm in the reactor R12.

The VFAs concentrations inside the system directly influence the pH and TA parameters. The pH is expected to reduce as the VFAs are produced during acidogenesis and acetogenesis. Later, when the

Table 5
Estimated process parameters during initial and final phase observed in the reactors.

Reactor nomenclature	Initial C/N ratio	Initial VS (g/L)	pH		EC (μS/cm)		TDS (mg/L)		TA (mg/L as $CaCO_3$)		VS removal (%)
			Initial	Final	Initial	Final	Initial	Final	Initial	Final	
R1	21.70	13.85	6.76 ± 0.22	6.95 ± 0.16	2245 ± 630	1437 ± 52	1595 ± 445	1025 ± 21	8313 ± 624	2125 ± 177	36 ± 10
R2	21.71	24.89	6.55 ± 0.01	6.98 ± 0.10	2125 ± 64	2296 ± 337	1520 ± 42	1663 ± 185	7375 ± 237	4333 ± 260	53 ± 5
R3	21.75	24.95	6.36 ± 0.04	6.93 ± 0.48	2300 ± 184	3097 ± 934	1625 ± 106	2030 ± 520	7438 ± 88	2833 ± 260	37 ± 7
R4	21.79	25.03	6.76 ± 0.68	7.13 ± 0.52	2110 ± 269	2510 ± 338	1500 ± 212	1776 ± 230	7688 ± 265	4500 ± 696	47 ± 2
R5	21.83	25.13	6.06 ± 0.01	7.04 ± 0.42	2525 ± 134	2646 ± 814	1795 ± 106	1790 ± 445	8938 ± 442	3500 ± 250	40 ± 10
R6	21.89	25.25	5.89 ± 0.01	6.37 ± 0.41	2760 ± 283	3317 ± 551	1990 ± 184	2187 ± 240	7500 ± 177	3167 ± 265	36 ± 6
R7	21.96	25.40	5.79 ± 0.15	6.98 ± 0.12	2580 ± 141	2310 ± 397	1840 ± 99	2053 ± 476	8188 ± 265	4542 ± 923	37 ± 14
R8	22.05	25.62	5.59 ± 0.13	6.96 ± 0.08	2650 ± 57	2435 ± 50	1900 ± 29	1735 ± 21	7438 ± 503	3333 ± 591	55 ± 5
R9	22.17	25.92	5.33 ± 0.12	6.69 ± 0.55	2665 ± 50	2673 ± 150	1905 ± 35	2160 ± 281	7000 ± 707	3375 ± 451	34 ± 4
R10	22.32	26.39	5.17 ± 0.13	6.92 ± 0.12	2910 ± 42	2696 ± 103	2065 ± 35	2210 ± 244	7563 ± 796	4042 ± 241	33 ± 12
R11	22.54	27.21	4.91 ± 0.19	6.06 ± 0.74	2955 ± 92	3097 ± 767	2110 ± 85	2093 ± 397	6812 ± 265	3083 ± 191	16 ± 9
R12	22.87	29.02	4.07 ± 0.03	5.61 ± 0.22	4775 ± 940	3310 ± 523	3380 ± 665	2325 ± 361	8000 ± 414	3833 ± 878	18 ± 10

methanogens consume these VFAs, the alkalinity of the slurry inside the reactor buffers the system neutralizing the pH (D' Silva et al., 2021a). The TA inside the reactors was also decreased over the period in all the reactors. The reactors' initial TA values were between 6813 ± 265 and 8938 ± 442 mg/L as $CaCO_3$. Similar observations were previously reported during anaerobic co-digestion of FVW with sugarcane bagasse with initial values between 5420 and 7520 mg/L as $CaCO_3$ (Vats et al., 2019). The neutralization of the pH requires the consumption of TA, and hence, the TA gradually decreases over time (Noonari et al., 2021; Vats et al., 2019). The consumption of TA was validated as the final TA was much lower than the initial TA values in the reactors. In particular, in reactor R8, the initial C/N ratio was found to be 22.05, and the initial pH was around 5.59 ± 0.13. Due to a suitable pH environment, the hydrolysis rate might have been initiated early. The succeeding reactions might have been supported in series providing stable operation and proper substrate degradation. This, in turn, might have contributed to the higher methane yield, which is directly involved with the VS reduction that occurred during the process (Gaur and Suthar, 2017).

The VS fed into the reactors was in the ranges of 13.85 g/L (R1) to 29.02 g/L (R12) at the experimental start. The fed VS content was reduced significantly, achieving a removal efficiency between 16 ± 9 and 55 ± 5 %. In the reactors R8 and R2, a significant reduction in VS were observed at the end of the digestion. Hence, the decrease in VS suggested that the organic matter was converted to intermediate VFAs for biomethane production in the reactors. The maximum VS removal in the reactors was observed in the order of R8 > R2 > R4 > R5 > R7 > R6 > R3 > R1 > R9 > R10 > R12 > R11. The results were similar to the VS removal of 17.73 to 56.57 % observed during the AcoD of activated sludge, anaerobic granular sludge and CD (Gaur and Suthar, 2017). The reactors R8 and R2 were much higher than the maximum VS removal, around 19.19 to 35.84 % observed during the AD of mustard residues, Chinese cabbage juice and residues. The biochemical reactions during the hydrolysis and acidogenesis process affect the VS reduction in the reactors. Hence, the improved VS reduction seems to be recorded in the reactors having a higher proportion of DFL than FVW. In addition, as the proportion of FVW increased, the amount of VFAs production might have escalated, reducing the pH inside the system and leading to decreased VS utilization by the microbes (Gaur and Suthar, 2017). The biomethane production and VS removal showed a similar trend, giving all the reactors proper mass balance.

3.3. Energy balance

The grinding of DFL substrate and pulverization of FVW substrate are energy-consuming processes. Hence, determining the energy balance of all the reactors is essential, considering the energy input and the energy output obtained. Table 6 represents the estimated energy balance results of all the reactors operated during the experimental run. The power consumption of the pulverization unit was higher than the grinding unit. As a result, the increase in FVW substrates increased the power input required for the reactor as the proportion of FVW substrates fed into the reactor increased. This was evident when the net energy output was looked upon. Irrespective of the net energy output, the maximum energy balance of 95.65 % was achieved in reactor R2, which was not fed with FVW substrate. Even though a positive energy balance was achieved in all the reactors, the net energy output is the main parameter that must be considered.

The overall net energy output of the reactor R8 with a co-substrate (DFL: FVW) ratio of 40:60 was higher with a value of 1.88 MJ compared to other reactors. The higher energy balance exhibited in all the reactors meant that the energy ratio was above value 1 (as shown in Table), which obtained a sufficient net energy output (Gaur and Suthar, 2017). Various researchers reported similar positive energy gains for different pretreatment techniques requiring input energy (mechanical and thermal) for AD of various biowastes, including lignocellulosic biomass and kitchen wastes (Ennouri et al., 2016; Gaur and Suthar,

Table 6
Energy balance estimations from the biomethane potential experiment.

Reactor configuration	Biogas produced (m^3)	Biomethane produced (m^3)	Methane content (%)	Total energy available (MJ)	Energy output (MJ) (assuming CHP and 10 % loss)	Heat output (MJ)	Electricity output (MJ)	Electricity output (kWh)	Energy input (pulverization and grinding, MJ)	Net energy output (MJ)	Energy balance (%)	Energy ratio
R1	0.04	0.02	39.87	0.56	0.51	0.28	0.22	0.06	–	0.51	100.00	–
R2	0.13	0.06	44.25	2.12	1.91	1.06	0.85	0.24	0.08	1.83	95.65	23.01
R3	0.09	0.04	44.96	1.47	1.33	0.74	0.59	0.16	0.09	1.24	93.58	15.57
R4	0.12	0.05	44.39	1.86	1.67	0.93	0.74	0.21	0.09	1.58	94.75	19.04
R5	0.10	0.05	46.78	1.62	1.46	0.81	0.65	0.18	0.09	1.36	93.76	16.02
R6	0.10	0.04	42.98	1.46	1.31	0.73	0.58	0.16	0.09	1.22	92.79	13.86
R7	0.09	0.04	43.77	1.43	1.29	0.72	0.57	0.16	0.10	1.19	92.27	12.93
R8	0.13	0.06	46.51	2.21	1.99	1.10	0.88	0.25	0.11	1.88	94.63	18.60
R9	0.11	0.05	44.63	1.78	1.60	0.89	0.71	0.20	0.12	1.48	92.72	13.73
R10	0.10	0.05	44.68	1.64	1.48	0.82	0.66	0.18	0.13	1.34	91.07	11.20
R11	0.06	0.02	37.51	0.85	0.77	0.43	0.34	0.09	0.16	0.61	79.35	4.84
R12	0.07	0.01	17.36	0.41	0.37	0.21	0.17	0.05	0.22	0.15	41.65	1.71

2017; Isha et al., 2021).

3.4. The future prospects, practical implications, and recommendations

This study evaluated the biomethane potential of anaerobic co-digestion of DFL, FVW and CD without any active inoculum. The study found massive potential for anaerobically co-digesting these substrates to obtain sustainable bioenergy production. Fig. 3 shows the future research directions, opportunities, and challenges of anaerobic co-digestion of DFL, FVW and CD. The batch study has been a preliminary investigation to determine the feasibility of the possible co-digestion strategy between these substrates. The potential development of this co-digestion strategy could be an approach suitable for all the regions since the DFL and FVW are available in most of the regions.

According to this batch study, the mixing ratio of 40:60 for DFL and FVW is the most suitable for increased methane production. About 1.93 % (% w/w) of water was saved through co-digestion at the optimal mixing ratio than mono-digestion of DFL. A more detailed investigation between the mixing of 50:50 to 30:70 with reduced gap intervals might give a more precise best ratio for enhanced productivity in terms of biomethane, synergistic effect points and extra water saved. Moreover, when the results of this study are considered for continuous reactor operation, there is a requirement of more parameters that must be considered, especially the HRT and feeding rate.

A preliminary assessment has been carried out in pilot-scale continuous reactors co-digesting the DFL and FVW using a mixing ratio of 40:60, which was already operated using FVW with an HRT of 45 d and feeding rate of 5 % TS, 7.5 % TS, and 10 % TS respectively, that showed that 5 % TS was the most suitable feeding rate for better energy productivity (data not shown). An HRT of 45 d was set since the T_{eff} for the prescribed mixing ratio of 40:60 was about 49 from this study, even though the co-digestion performance at lower HRT could be investigated further. The feeding rate of 5 % TS was earlier reported as optimal for the lignocellulosic biomass in the continuous reactor while co-digesting rice straw and de-oiled rice bran in the author's laboratory (Jha et al., 2021). The non-utilization of an active inoculum was to analyze the opportunity to reduce the start-up period. The lowering of the start-up may support the initialization of energy output without waiting for more time to start the feeding.

However, there could be an investigation over the biomethane potential of DFL and FVW with an active inoculum to investigate its effect on HRT. A more intensive long-term study in a continuous reactor is required to examine the seasonal variations of energy production with respect to ambient temperature. The variation in process parameters, mass balance and digestate quality at steady-state conditions must be analyzed. The optimization of operation, maintenance, material handling, techno-economics, and life cycle analysis must be investigated. This could determine whether this anaerobic co-digestion strategy is viable for society irrespective of the regions.

With less pretreatment requirement, less energy input is a significant factor in reducing the capital cost and less carbon footprint. This must be investigated further, considering the full-scale applications. The World Biogas Association endorses establishing full-scale AD plants, which could achieve a local circular economy and reduce the greenhouse gases (predominantly methane) that AD is known for (World Biogas Association, 2021). Co-digesting FVW and DFL could benefit developing countries like India to achieve substantial renewable energy power production (Bandgar et al., 2021). The co-digestion of DFL and FVW could remarkably enhance the dissemination of the biogas technology more efficiently, tackling the challenges. However, a proper recording of leaf shedding by each tree species and the calculation and optimization of surplus leaf biomass does not hinder the natural carbon cycle. Moreover, the shed leaves might contain sand and other inert materials that may disrupt the AD process. This requires the development of proper collection and segregation techniques.

4. Conclusions

This study aimed to investigate the biomethane potential of anaerobic co-digestion of DFL, FVW, and CD. The reactor fed with a DFL: FVW mixing ratio of 40:60 showed an enhanced methane yield of 388 ± 131 mL/g VS_{input}. The reactor showed a good biodegradability of 77 % for biomethane production and a CPI value of 1.04. The stabilized process parameters have enhanced the methane yield. Thus, the AcoD of DFL, FVW, and CD possesses a vast prospect for full-scale applications. The study summarizes that there is a requirement for further research on the development of the proposed AcoD concept.

CRediT authorship contribution statement

Tinku Casper D' Silva: Conceptualization, Methodology, Investigation, Writing – original draft, Data curation, Formal analysis. **Adya**

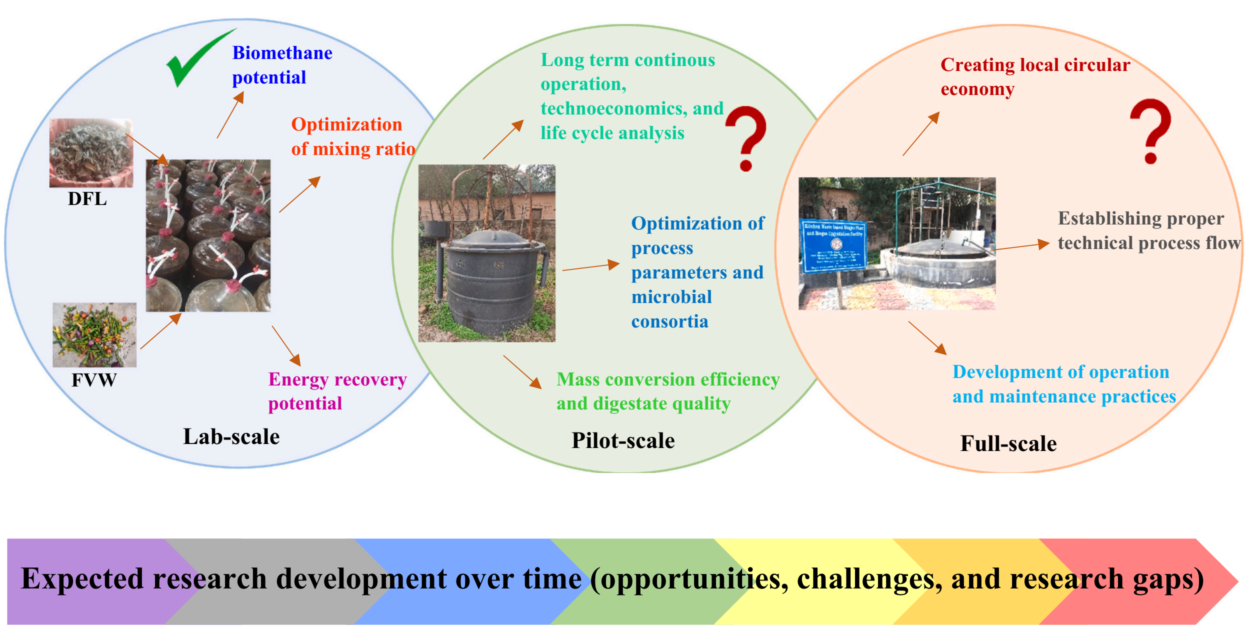

Fig. 3. Future research directions for anaerobic co-digestion of DFL, FVW and CD.

Isha: Methodology, Investigation, Writing – review & editing. **Srishti Verma:** Investigation, Writing – review & editing. **Ganesh Shirsath:** Writing – review & editing. **Ram Chandra:** Supervision, Visualization, Resources, Writing – review & editing. **Virendra Kumar Vijay:** Supervision, Resources. **Paruchuri M.V. Subbarao:** Supervision. **Kornél L. Kovács:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data has been given annexures.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2022.101189.

References

Ambrose, H.W., Philip, L., Suraishkumar, G.K., Karthikaichamy, A., Sen, T.K., 2020. Anaerobic co-digestion of activated sludge and fruit and vegetable waste: Evaluation of mixing ratio and impact of hybrid (microwave and hydrogen peroxide) sludge pre-treatment on two-stage digester stability and biogas yield. J. Water Process Eng. 37, 101498 https://doi.org/10.1016/j.jwpe.2020.101498.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927–934. https://doi.org/10.2166/wst.2009.040.

APHA, AWWA,WEF, 2012. Standard Methods for Examination of Water and Wastewater. Washingt. Am. Public Heal. Assoc.

Arhoun, B., Villen-Guzman, M., Gomez-Lahoz, C., Rodriguez-Maroto, J.M., Garcia-Herruzo, F., Vereda-Alonso, C., 2019. Anaerobic co-digestion of mixed sewage sludge and fruits and vegetable wholesale market waste: composition and seasonality effect. J. Water Process Eng. 31, 100848 https://doi.org/10.1016/j.jwpe.2019.100848.

Awoyale, A.A., Lokhat, D., 2021. Experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in bioethanol production. Sci. Rep. 11, 1–16. https://doi.org/10.1038/s41598-020-78105-8.

Baghel, P., Sakhiya, A.K., Kaushal, P., 2021. Influence of temperature on slow pyrolysis of Prosopis Juliflora: an experimental and thermodynamic approach. Renew. Energy 185. https://doi.org/10.1016/j.renene.2021.12.053.

Bandgar, P.S., Jain, S., Panwar, N.L., 2021. Current status, opportunities and challenges in anaerobic digestion in Indian context: an overview. Bioresour. Technol. Rep. 16, 100830 https://doi.org/10.1016/j.biteb.2021.100830.

Bres, P., Beily, M.E., Young, B.J., Gasulla, J., Butti, M., Crespo, D., Candal, R., Komilis, D., 2018. Performance of semi-continuous anaerobic co-digestion of poultry manure with fruit and vegetable waste and analysis of digestate quality: a bench scale study. Waste Manag. 82, 276–284. https://doi.org/10.1016/j.wasman.2018.10.041.

Chandra, R., Takeuchi, H., Hasegawa, T., Kumar, R., 2012. Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. Energy 43, 273–282. https://doi.org/10.1016/j.energy.2012.04.029.

Chaudhary, V.P., Chandra, R., Denis, D.M., Isha, A., D'Silva, T.C., 2022. Agri-biomass-based bio-energy supply model: an inclusive sustainable and circular economy approach for a self-resilient rural India. Biofuels Bioprod. Biorefin. 1–13. https://doi.org/10.1002/bbb.2373.

D' Silva, T.C., Isha, A., Chandra, R., Vijay, V.K., Subbarao, P.M.V., Kumar, R., Chaudhary, V.P., Singh, H., Khan, A.A., Tyagi, V.K., Kovács, K.L., 2021. Enhancing methane production in anaerobic digestion through hydrogen assisted pathways – a state-of-the-art review. Renew. Sustain. Energy Rev. 151, 111536 https://doi.org/10.1016/j.rser.2021.111536.

D' Silva, T.C., Verma, S., Magdaline, R.M., Chandra, R., Khan, A.A., 2021. Environmental resilience and sustainability through green technologies: a case evidence from rural coastal India. Environ. Eng. Res. 27 https://doi.org/10.4491/eer.2021.262, 210262–0.

Darmawan, S., Wistara, N.J., Pari, G., Maddu, A., Syafii, W., 2016. Characterization of lignocellulosic biomass as raw material for the production of porous carbon-based materials. BioResources 11 (2), 3561–3574.

Datta, R., 1981. Acidogenic fermentation of lignocellulose–acid yield and conversion of components. Biotechnol. Bioeng. https://doi.org/10.1002/bit.260230921.

Dumas, C., Silva Ghizzi Damasceno, G., Abdellatif, B., Carrère, H., Steyer, J.P., Rouau, X., 2015. Effects of grinding processes on anaerobic digestion of wheat straw. Ind. Crops Prod. 74, 450–456. https://doi.org/10.1016/j.indcrop.2015.03.043.

Ebner, J.H., Labatut, R.A., Lodge, J.S., Williamson, A.A., Trabold, T.A., 2016. Anaerobic co-digestion of commercial food waste and dairy manure: characterizing biochemical parameters and synergistic effects. Waste Manag. 52, 286–294. https://doi.org/10.1016/j.wasman.2016.03.046.

Ennouri, H., Miladi, B., Diaz, S.Z., Güelfo, L.A.F., Solera, R., Hamdi, M., Bouallagui, H., 2016. Effect of thermal pretreatment on the biogas production and microbial communities balance during anaerobic digestion of urban and industrial waste activated sludge. Bioresour. Technol. 214, 184–191. https://doi.org/10.1016/j.biortech.2016.04.076.

Fernandez, H.C., Buffiere, P., Bayard, R., 2022. Understanding the role of mechanical pretreatment before anaerobic digestion: lab-scale investigations. Renew. Energy 187, 193–203. https://doi.org/10.1016/j.renene.2022.01.067.

Fonoll, X., Astals, S., Dosta, J., Mata-Alvarez, J., 2015. Anaerobic co-digestion of sewage sludge and fruit wastes: evaluation of the transitory states when the co-substrate is changed. Chem. Eng. J. 262, 1268–1274. https://doi.org/10.1016/j.cej.2014.10.045.

Gao, M., Zhang, L., Liu, Y., 2020. High-loading food waste and Blackwater anaerobic co-digestion: maximizing bioenergy recovery. Chem. Eng. J. 394, 124911 https://doi.org/10.1016/j.cej.2020.124911.

Gaur, R.Z., Suthar, S., 2017. Anaerobic digestion of activated sludge, anaerobic granular sludge and cow dung with food waste for enhanced methane production. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2017.06.201.

Gaur, R.Z., Khan, A.A., Suthar, S., 2017. Effect of thermal pre-treatment on co-digestion of duckweed (Lemna gibba) and waste activated sludge on biogas production. Chemosphere 174, 754–763. https://doi.org/10.1016/j.chemosphere.2017.01.133.

Gerardi, M.H., 2003. The Microbiology of Anaerobic Digesters. John Wiley & Sons, Inc, Hoboken, New Jersey. https://doi.org/10.1002/0471468967.

Hou, T., Zhao, J., Lei, Z., Shimizu, K., Zhang, Z., 2020. Synergistic effects of rice straw and rice bran on enhanced methane production and process stability of anaerobic digestion of food waste. Bioresour. Technol. 314, 123775 https://doi.org/10.1016/j.biortech.2020.123775.

Isha, A., Kumar, S., Jha, B., Subbarao, P.M.V., Chandra, R., Vijay, V.K., 2020. Development of stabilization methods using a pilot scale anaerobic digester for seasonal variations in kitchen wastes for improved methane production with zero breakdowns. Clean. Eng. Technol. 1, 100015 https://doi.org/10.1016/j.clet.2020.100015.

Isha, A., D'Silva, T.C., Subbarao, P.M.V., Chandra, R., Vijay, V.K., 2021. Stabilization of anaerobic digestion of kitchen wastes using protein-rich additives: study of process performance, kinetic modelling and energy balance. Bioresour. Technol. 337, 125331 https://doi.org/10.1016/j.biortech.2021.125331.

Jha, B., Isha, A., Trivedi, A., Chandra, R., Subbarao, P.M.V., 2021. Anaerobic co-digestion of rice straw and de-oiled rice bran for biomethane production. Energy Rep. 7, 704–710. https://doi.org/10.1016/j.egyr.2021.01.032.

Khan, S.A., D' Silva, T.C., Kumar, S., Chandra, R., Vijay, V.K., Misra, A., 2021. Mutually trading off biochar and biogas sectors for broadening biomethane applications: a comprehensive review. J. Clean. Prod. 318, 128593 https://doi.org/10.1016/j.jclepro.2021.128593.

Khayum, N., Anbarasu, S., Murugan, S., 2018. Biogas potential from spent tea waste: a laboratory scale investigation of co-digestion with cow manure. Energy 165, 760–768. https://doi.org/10.1016/j.energy.2018.09.163.

Kim, Jaai, Kim, Jinsu, Lee, C., 2019. Anaerobic co-digestion of food waste, human feces, and toilet paper: methane potential and synergistic effect. Fuel 248, 189–195. https://doi.org/10.1016/j.fuel.2019.03.081.

Kumar, S., Gandhi, P., Yadav, M., Paritosh, K., Pareek, N., Vivekanand, V., 2019. Weak alkaline treatment of wheat and pearl millet straw for enhanced biogas production and its economic analysis. Renew. Energy 139, 753–764. https://doi.org/10.1016/j.renene.2019.02.133.

Kumar, S., D'Silva, T.C., Chandra, R., Malik, A., Vijay, V.K., Misra, A., 2021. Strategies for boosting biomethane production from rice straw: a systematic review. Bioresour. Technol. Rep. 15, 100813 https://doi.org/10.1016/j.biteb.2021.100813.

Labatut, R.A., Angenent, L.T., Scott, N.R., 2011. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour. Technol. 102, 2255–2264. https://doi.org/10.1016/j.biortech.2010.10.035.

Lesteur, M., Bellon-Maurel, V., Gonzalez, C., Latrille, E., Roger, J.M., Junqua, G., Steyer, J.P., 2010. Alternative methods for determining anaerobic biodegradability:

a review. Process Biochem. 45, 431–440. https://doi.org/10.1016/j.procbio.2009.11.018.

Muhammad, M.B., Chandra, R., 2021. Enhancing biogas and methane production from leaf litter of neem by co-digestion with vegetable waste: focus on the effect of tannin. Biomass Bioenergy 147, 106007. https://doi.org/10.1016/j.biombioe.2021.106007.

Nielfa, A., Cano, R., Fdz-Polanco, M., 2015. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. Biotechnol. Rep. 5, 14–21. https://doi.org/10.1016/j.btre.2014.10.005.

Nieto-Gligorovski, L., Net, S., Gligorovski, S., Zetzsch, C., Jammoul, A., D'Anna, B., George, C., 2008. Interactions of ozone with organic surface films in the presence of simulated sunlight: impact on wettability of aerosols. Phys. Chem. Chem. Phys. 10, 2964–2971. https://doi.org/10.1039/b717993f.

Noonari, A.A., Shah, A.R., Mirjat, N.H., Anh, T., 2021. Various pretreatments of canola straw with hydrogen peroxide , calcium hydroxide, silica, and Pleurotus ostreatus to improve methane yield through anaerobic co - digestion. Biomass Convers. Biorefinery. https://doi.org/10.1007/s13399-021-02226-x.

Paes, J.L., Alves, T.B.S., Silva, L.D.B., Marques, A.D.S., Dias, V.R.S., 2020. Use of inoculum in biodigesters with cattle manure under conventional and organic production systems. Eng. Agrícola 40, 146–153. https://doi.org/10.1590/1809-4430-Eng.Agric.v40n2p146-153/2020.

Pagés-Díaz, J., Pereda-Reyes, I., Taherzadeh, M.J., Sárvári-Horváth, I., Lundin, M., 2014. Anaerobic co-digestion of solid slaughterhouse wastes with agro-residues: synergistic and antagonistic interactions determined in batch digestion assays. Chem. Eng. J. 245, 89–98. https://doi.org/10.1016/j.cej.2014.02.008.

Pannase, A., Lajewar, E.J., Khandeshwar, S., 2020. Performance analysis of anaerobic digester for food and dairy industries. Int. Res. J. Eng. Technol. 7, 603–605.

Singh, R., Tiwari, S., Srivastava, M., Shukla, A., 2014. Microwave assisted alkali pretreatment of rice straw for enhancing enzymatic digestibility. J. Energy. https://doi.org/10.1155/2014/483813.

Szilágyi, Á., Bodor, A., Tolvai, N., Kovács, K.L., Bodai, L., Wirth, R., Bagi, Z., Szepesi, Á., Markó, V., Kakuk, B., Bounedjoum, N., Rákhely, G., 2021. A comparative analysis of biogas production from tomato bio-waste in mesophilic batch and continuous anaerobic digestion systems. PLoS One 16, 1–17. https://doi.org/10.1371/journal.pone.0248654.

Tang, B., He, M., Dong, Y., Liu, J., Zhao, X., Wang, C., Wu, K., Yin, F., Zhang, W., 2020. Effects of different forms of vegetable waste on biogas and methane production performances in a batch anaerobic digestion reactor. Energy Sources, Part A 00, 1–11. https://doi.org/10.1080/15567036.2020.1818003.

Vats, N., Khan, A.A., Ahmad, K., 2019. Effect of substrate ratio on biogas yield for anaerobic co-digestion of fruit vegetable waste & sugarcane bagasse. Environ. Technol. Innov. https://doi.org/10.1016/j.eti.2019.01.003.

Vijay, V., Subbarao, P.M.V., Chandra, R., 2021. An evaluation on energy self–sufficiency model of a rural cluster through utilization of biomass residue resources: a case study in India. Energy Clim. Chang. 2, 100036 https://doi.org/10.1016/j.egycc.2021.100036.

Wang, X., Li, Z., Bai, X., Zhou, X., Cheng, S., Gao, R., Sun, J., 2018. Study on improving anaerobic co-digestion of cow manure and corn straw by fruit and vegetable waste: methane production and microbial community in CSTR process. Bioresour. Technol. 249, 290–297. https://doi.org/10.1016/j.biortech.2017.10.038.

World Biogas Association, 2021. Anaerobic digestion dangerously overlooked as immediate mitigation solution – WBA warns. https://bioenergyinternational.com/opinion-commentary/anaerobic-digestion-dangerously-overlooked-as-immediate-mitigation-solution-wba-warns. (Accessed 17 February 2022).

Yang, G., Hu, Y., Wang, J., 2019. Biohydrogen production from co-fermentation of fallen leaves and sewage sludge. Bioresour. Technol. 285, 121342 https://doi.org/10.1016/j.biortech.2019.121342.

Yong, Z., Dong, Y., Zhang, X., Tan, T., 2015. Anaerobic co-digestion of food waste and straw for biogas production. Renew. Energy 78, 527–530. https://doi.org/10.1016/j.renene.2015.01.033.

Yoon, Y.M., Kim, S.H., Oh, S.Y., Kim, C.H., 2014. Potential of anaerobic digestion for material recovery and energy production in waste biomass from a poultry slaughterhouse. Waste Manag. 34, 204–209. https://doi.org/10.1016/j.wasman.2013.09.020.

Zamanzadeh, M., Hagen, L.H., Svensson, K., Linjordet, R., Horn, S.J., 2017. Biogas production from food waste via co-digestion and digestion- effects on performance and microbial ecology. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-15784-w.