



Enhancing methane production by anaerobic co-digestion of extruded organic wastes from slaughterhouse and vegetable market in batch and continuous processes

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Abstract

The organic wastes generated from centralized wholesale markets from urban centres are predominantly disposed in dumpsites/landfills. Although anaerobic digestion (AD) of wastes is a promising option for producing bio-fuel, the key barrier is the limitation in the waste conversion efficiency that demands the need for the organic waste pretreatment. Hence, the extrusion pre-treatment of wastes prior to AD of slaughterhouse wastes (ESHW) with mixed vegetable, fruit and flower market wastes (EVMW), from a centralized-wholesale market in an urban area, was studied in batch and continuous AD processes. Batch experiments were carried out in mono- and co-digestion modes of extruded wastes. The results of the batch study revealed maximum specific methane yield of 354.9 mL_N/g oDM during anaerobic co-digestion of extruded slaughterhouse wastes with mixed vegetable, fruit and flower market wastes in 1:3 proportion (ESHW/EVMW). Based on these results, lab-scale continuous reactor study (15 L) at varying organic loading rates (OLR) was carried out and the maximum biogas yield of 618.5 mL_N/g oDM with a methane content of 62.6% and energy potential of 12.2 MJ/kg DM was obtained for extruded wastes at an OLR of 2.5 g oDM/L/d (E) when compared with non-extruded wastes that showed biogas potential of 503.7 mL_N/g oDM (with energy potential of 10.2 MJ/kg DM). The outcome of the study revealed that extrusion pretreatment along with anaerobic co-digestion resulted in an increase in the biogas production therein showing enhancement in green energy production during anaerobic co-treatment of centralized wholesale market and slaughterhouse wastes in urban centres.

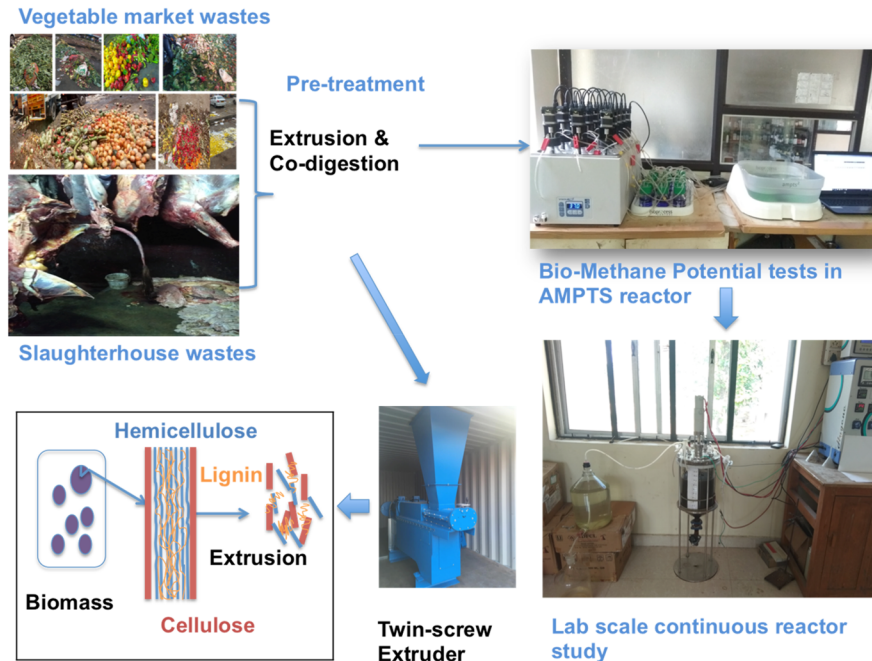
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Graphic Abstract



Keywords Biogas · Slaughterhouse · Vegetable market wastes · Urban centres · Solid waste · Extrusion pre-treatment · Continuous anaerobic reactor

Introduction

Currently, more than half of humanity lives in urban areas, and it is estimated that 60% of global population will be in cities by 2030. It is also pertinent to mention that cities occupy only two per cent of the world's landmass but consume over two-thirds of the world's energy and account for more than 70 per cent of global CO₂-emissions. There are 33 Mega-Cities (> 10 million population) in the world where 17 cities are located in the Asia Pacific region. In 2030, it is projected that there will be 43 Mega-Cities globally and among which, 35 Mega-Cities will be in the Asia Pacific region. With this rapid urbanization rate, cities are at the forefront to adopt innovative, sustainable energy solutions.

Rapid urbanization results in generation of municipal solid waste and domestic wastewater which needs to be treated and disposed in safe manner. In most of the major cities in developing countries, the solid wastes generated from urban areas are disposed in open dumpsites or landfills and still pose a threat to the environment. Furthermore, methane emission from solid waste dumpsites/landfills is one among the major anthropogenic sources of GHG that contribute about 11% of the global anthropogenic GHG emissions (Singh et al. 2018).

India is one among the major countries that share 7% of global GHG emissions contributing about 2299 million tons of carbon dioxide (CO₂) in 2018 which further increased to 2616 million tons of CO₂ in 2019 (IEA 2018; Statistics data 2019). During 2019–2020, the emissions have reduced by 30 million tons for the first time in the last four decades due to COVID pandemic lock down (CO₂ emissions data 2020).

Also, in India, only 70–75% of the Municipal Solid Waste (MSW) generated is being collected in which 22–28% is being treated through composting, recycling and landfilling, whereas the rest is disposed in dumpsites (PIB 2016; Singh et al. 2018). The composition of MSW is primarily shared by organic fractions (about 40–60%) that include wastes from households, vegetable, fruit and flower markets, slaughterhouses, fish markets, etc. (Sharma and Jain 2019). Out of these organic fractions, solid waste streams like wholesale vegetable, fruit and flower market wastes, and slaughterhouse wastes are the major point sources of organic waste generation in urban centres (Patinvoh et al. 2018; Jha et al. 2008). These wastes from wholesale-centralized markets pose a great challenge for the management of waste generated from various urban centres in India. On the other hand, vegetable, fruit and flower market wastes, and slaughterhouse wastes are rich in the volatile matter and moisture content hence shows

high suitability for clean and green energy generation through anaerobic digestion (Scano et al. 2014). Thus, utilization of the waste for green energy/biofuel production (biogas) through anaerobic digestion not only helps to reduce GHG emissions but also meets the rising energy demand thereby providing a win–win situation. However, the efficiency of anaerobic mono-substrate (individual organic fractions of MSW) digestion is limited due to the presence of AD process inhibitors such as volatile fatty acids (VFA) accumulation, ammonia inhibition, sulphide inhibition, poor biodegradability of waste, etc. (Zhu et al. 2018; Edwiges et al. 2018; Cuetos et al. 2010).

Zhu et al. (2018) observed the decrease in methane production activity from 38.4 to 15.2% while the free ammonia concentration increased from 325 to 695 mg/L during AD of poultry litter. Edwiges et al. (2018) observed volatile fatty acid accumulation with a decrease in the methane yield while OLR was increased beyond 3.0 g oDM/L/d during continuous AD of vegetable and fruit wastes. Cuetos et al. (2010) observed long chain fatty acids inhibition (with a concentration of 620 mg/L) with low fat removal efficiency (about 22%) during AD of slaughterhouse wastes. Anaerobic co-digestion of different substrates together was reported to balance out these process inhibitions by enhancing the distribution of nutrients, balancing pH, enhanced biodegradability, etc., to enhance the clean energy production (Song et al. 2019; Alvarez et al. 2010).

In addition to anaerobic co-digestion, pretreatment of wastes further enhances the rate of biodegradation and energy production. Among the series of reactions involved in the AD process, i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis, hydrolysis is considered to be the rate-limiting process since it greatly influences the wastes' degradation efficiency (Karuppiyah and Azariah 2019). The delay in hydrolysis is often a result of poor degradation of lignin compounds that embeds the cellulose and hemicellulose compounds, thereby preventing the microbial access to the biomass (Zheng et al. 2019). Hence, pretreatment of wastes, prior to AD process would result in the breakdown of the complex biomass structure therein increasing the microbial accessibility to the biomass for enhanced enzymatic hydrolysis (Kucharska et al. 2018). Existing pretreatment studies on vegetable, fruit, flower and slaughterhouse wastes showed enhancement in methane yield as a result of various pretreatments such as acid and alkaline hydrolysis, hydrothermal pretreatment, steam explosion, ultrasonic and mechanical pretreatments. For instance, an increase in the methane yield from 217.8 to 283.4 mL_N/g oDM was obtained as a result of acid hydrolysis of potato peels (Achinah et al. 2019). Similarly, an increase in the methane yield from 280.9 to 326.0 mL_N/g oDM was obtained as a result of hydrothermal pretreatment of fruit and vegetable wastes. Further, alkaline pretreatment of tomato wastes prior to AD

showed an increase in the methane yield from 330.0 to 335.0 mL_N/g oDM (Calabrò et al. 2015).

Similar to these pretreatments, extrusion is one among the pretreatments that is applied prior to the AD of wastes for enhanced energy production from biomass. Extrusion process has been considered as a novel pre-treatment prior to AD since it encompasses multiple operations in one unit (Lehmann 2009). Once the substrate is fed into the extruder, the structure of biomass changes due to the heat generated from friction, mixing and shearing which results in defibrillation and size reduction in the fibres (Pilarski et al. 2016). This increases the accessibility of the microorganisms within the AD system, which in turn enhances the hydrolysis of biomass as a result of extrusion pre-treatment (Karunanithy and Muthukumarappan 2011). Hence, extrusion as a pre-treatment helps to overcome this issue thereby reducing the hindrance to the accessibility by breaking up the biomass structure (Zheng and Rehmann 2014). Further, it is found to increase the methane yield of biomass by about 18–72%; however existing studies on extrusion pre-treatment primarily focused on energy crops for energy generation, which showed greater efficiencies for lignocellulosic biomass (Pilarski et al. 2016; Chen et al. 2014). Thus, it is understood from the literature that pretreatment processes such as acid and alkaline hydrolysis, hydrothermal pretreatment, steam explosion, ultrasonic, and mechanical processes have been intensively reported on vegetable, fruit, flower and slaughterhouse wastes prior to the AD process. Nevertheless, to the best of our knowledge, the effect of extrusion pretreatment and anaerobic co-digestion of vegetable, fruit, flower market wastes with slaughterhouse wastes in both batch and continuous scale are not investigated. Hence, this study predominantly focuses on investigating the impact of extruded wastes on the anaerobic co-digestion of slaughterhouse and vegetable market wastes for enhanced energy production.

Herein, based on the identified research gaps, for the first time, the combined effect of extrusion pretreatment and anaerobic co-digestion for enhanced methane production during AD of vegetable, fruit, flower market wastes with slaughterhouse wastes in both batch and continuous AD study is studied. Furthermore, the impact of optimal substrate management of wastes generating from centralized slaughterhouses and wholesale vegetable, fruit and flower markets from urban centres are also investigated.

Materials and methods

Materials

The Vegetable, Fruit and Flower market wastes (VMW) were collected from one of Asia's largest centralized horticultural market at Koyambedu, Chennai, India. Sampling

was carried out following sample sorting and preparation standards by LAGA PN98 protocol (2016) and Weichgrebe et al. (2017). Once VMW samples were brought to the laboratory, small portion of uniformly mixed wastes was minced using a lab-scale mincer (La Mineva, A/E12-05). The minced wastes were then mixed in the waste generation proportions in the wholesale market with a vegetable waste share of 45 wt.%, banana peduncle of 30 wt.%, fruit waste of 23 wt.% and flower waste of 2 wt.% (Mozhiarasi et al. 2019). These minced wastes were well mixed and stored at 4 °C for further use.

In the slaughterhouses, during cattle slaughtering, it was found that bones, lungs, heads and hooves, carcass and hides shares about 78.6%, which are further utilized, whereas the rest 21.4% is left over as wastes from slaughterhouses with shares of ruminal contents (83 wt.%) and blood (17 wt.%). The slaughterhouse wastes were collected from the largest slaughterhouse in Chennai, India. Similar to VMW, slaughterhouse wastes (SHW) were minced and stored for further experimentation.

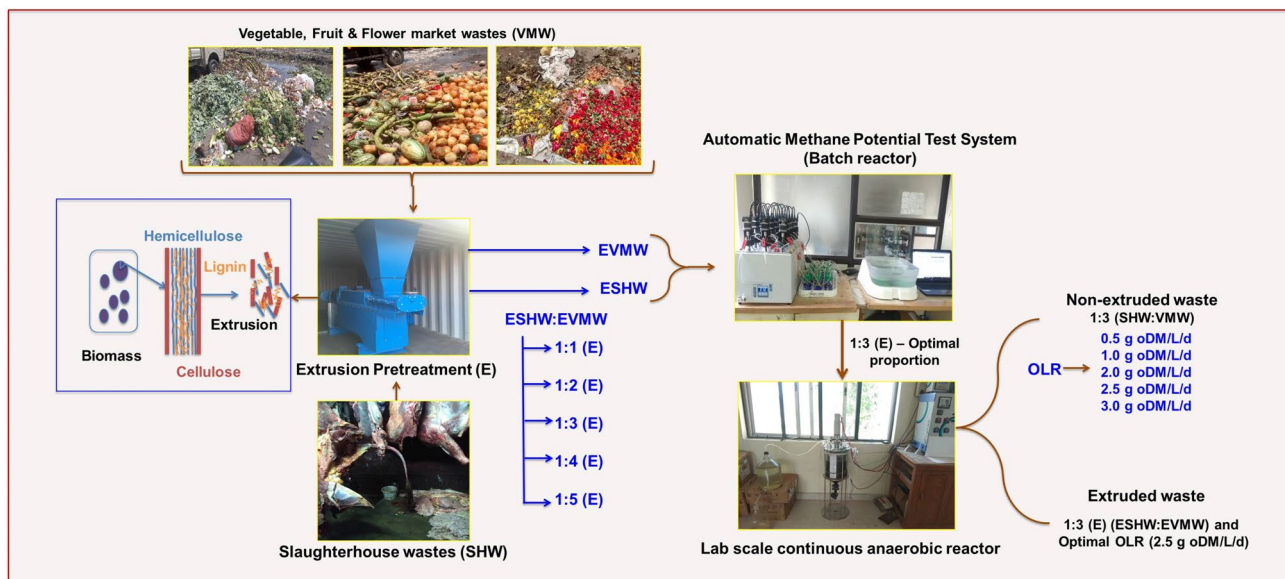
About 100 kg of each waste (such as vegetable, fruit, flower, ruminal contents and blood) was extruded separately. The extrusion was carried out in a pilot-scale twin-screw Bio-Extruder® (capacity of 200 kg/h) by Lehmann-UMT GmbH (Model: MSZ B 22e), Germany (Lehmann 2009). The extruded VMW (EVMW) was mixed based on the waste generation proportions in the respective markets. Similarly, the extruded SHW (ESHW) was also mixed. The inoculum for AD tests was collected from the anaerobic digester of a nearby sewage treatment plant, which has a pH of 7.2, with dry matter (DM) and organic dry matter (oDM) of 3.8 and 2.4 wt.%, respectively.

Analytical methods

The wastes were characterized for moisture content, DM, oDM, pH and ammoniacal nitrogen according to APHA standard methods (APHA 1998). The protein, lipid and carbohydrates contents were estimated by following Bradford (1976), Frings and Dunn (1970) and Dubois et al. (1956), respectively. Fibre analyses (Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF)) were done according to Van Soest et al. (1991). Hemicellulose (HC) was measured by the differences between NDF and ADF. The elemental analysis was carried out according to ASTM D5373 standards using an elemental analyzer (EA 3000, Euro Vector) (ASTM 2014). Volatile Fatty Acids (VFA) and alkalinity were estimated by following the method described by Dilallo and Albertson (1961).

Batch AD study

The schematic of the current study with anaerobic digestion process (batch and continuous) of vegetable, fruit and flower with slaughterhouse wastes is shown in Scheme 1. Anaerobic digestion of EVMW, ESHW alone and ESHW with EVMW in different proportions from 1:1 (E) (ESHW:EVMW) to 1:5 (E) (ESHW:EVMW) were carried out, using Automatic methane potential Test System (AMPTS II, Bioprocess control) by following the protocol described by VDI 4630 standards (VDI-Guideline 4630 2006). Following this protocol, the study was carried out at mesophilic conditions (37 °C) in 600 mL reactors with an effective volume of 400 mL with Substrate/Inoculum ratio (S/I) of 0.5 (g oDM/g oDM). The Specific Methane Yield (SMY) obtained from the AD tests



Scheme 1 Block diagram of anaerobic digestion of wastes (Batch and Continuous processes)

was presented as normalized volume of methane produced/gram of organic dry matter (oDM) added ($\text{mL}_N/\text{g oDM}$). All the experiments were carried out in triplicates. The oDM reduction, pH, VFA and alkalinity were measured after batch AD tests.

Continuous AD study

The continuous reactor study was carried out in a lab-scale continuous reactor made of glass of 15 L capacity with a working volume of 10 L. The reactor has a stirrer (maintained at 30 RPM), pH probe, temperature control (maintained at 37 °C), gas outlet, feed inlet and outlet arrangements. The daily biogas production was monitored using water displacement method and the methane share was measured by allowing the biogas to pass through base solution (3 M NaOH) with thymolphthalein indicator during the last 5 days at each loading rates from 31st to 35th day. Subsequently, the average methane content was presented.

The continuous AD studies of non-extruded SHW with VMW at optimal co-digestion proportion, obtained from the results of batch tests, were carried out at varying organic loading rates as 0.5, 1.0, 2.0, 2.5 and 3.0 g of organic dry matter per litre per day (g oDM/L/d) at HRT of 25 days in order to optimize the organic loading rate. Initially, the continuous reactor was started at a loading rate of 0.5 g oDM/L/d followed by the subsequent increase in the next loading rate, with a change in every 35 days, until a final loading of 3.0 g oDM/L/d is reached. At each loading rate, for the first 30 days, the Specific Biogas Yield (SBY) was measured and in the later 5 days, the specific methane yield was measured,

and thus each loading was operated for 35 days. Hence, for the non-extruded wastes, the continuous reactor was operated for 175 days (5 loadings \times 35 days).

Following this, the continuous AD study of extruded SHW with VMW was carried out at the optimal loading rate obtained from continuous AD study of non-extruded proportions of SHW with VMW at varying loading rates from 0.5 to 3.0 g oDM/L/d. Hence, for extruded wastes, the continuous reactor was operated from 176th to 210th day. Thus, the continuous reactor was operated for 210 days with 6 loading rates and the results are presented.

In addition to biogas production and methane share, pH, VFA/Alkalinity ratio, ammoniacal nitrogen and oDM degradation (%) were monitored to evaluate the reactor stability and performance at varying organic loading rates. Further, the Specific Methane Yield values of extruded and non-extruded wastes at varying organic loading rates were changed into energy potentials using the gross calorific value (GCV) of methane (39.8 MJ/m^3) (Hahne, 2010). The results of energy potentials are presented on a dry matter basis.

Results and discussion

Characterization of wastes

The characteristics of wastes, i.e. ESHW, EVMW, 1:1(E), 1:2(E), 1:3(E), 1:4(E), and 1:5(E) in terms of pH, DM, oDM, moisture, carbohydrate, protein, lipid, NDF, ADF, HC and C/N ratio are presented in Table 1.

From Table 1, it was found that the pH of EVMW lies in the acidic range of 5.2, whereas the pH of ESHW remains as

Table 1 Waste characterization

Parameter	ESHW	EVMW	1:1(E)	1:2(E)	1:3(E)	1:4(E)	1:5(E)
pH	6.94	5.21	6.34	6.01	5.94	5.77	5.56
DM (%)	22.19	10.32	15.97	13.98	13.02	12.16	11.86
oDM (%)	17.61	8.17	12.46	10.99	10.29	9.72	9.54
oDM (%DM)	79.35	79.17	78.04	78.61	79.04	79.94	80.46
Moisture (%)	77.81	89.68	84.03	86.02	86.98	87.84	88.14
Carbohydrate*	440.03	618.26	537.96	566.36	583.51	595.30	603.71
Protein*	191.56	121.25	162.64	149.82	143.16	139.45	136.35
Lipid*	101.79	31.42	64.30	57.12	51.86	47.66	44.04
NDF*	280.12	399.58	331.72	349.47	356.05	362.85	369.03
ADF*	204.74	240.27	215.18	220.27	224.06	228.12	226.50
HC*	75.37	159.31	116.55	129.20	132.00	134.73	142.52
C (% DM)	42.04	36.92	38.47	39.92	38.17	37.98	37.01
N (% DM)	1.64	1.83	1.67	1.74	1.78	1.80	1.79
H (% DM)	11.87	7.72	9.63	8.94	8.54	8.41	8.13
O (% DM)	44.45	53.53	50.24	49.40	51.51	51.81	53.07
C/N	25.63	20.17	23.09	22.95	21.48	21.12	20.65

*g/kg of DM; All values in the table are mean of triplicates

6.9, which is in close proximity to the optimal limit required for AD in the range of 6.8–7.2 (Cioabla et al. 2012). However, the pH of all co-digested wastes lies within the range of 5.6–6.3 with a drop in the pH in concurrence with the EVMW proportion in the co-digestion mixture. The oDM share of DM ranged between 78.0 and 80.5% with the variations in the moisture content ranging between 77.8 and 89.7%. Thus, the presence of high moisture and oDM content showed the substrates' suitability for the AD process (Morales-Polo et al. 2018).

While comparing EVMW and ESHW, the carbohydrate content is higher in EVMW, whereas ESHW contains higher amounts of lipid and protein content. This is the reason for the increase in the carbohydrate content in the co-digestion mixtures from 1:1(E) to 1:5(E) in the range of 53.8–60.4% of DM. Thus, carbohydrates are the predominant macromolecules in the DM. In carbohydrates, the major portion (61.0–64.6%) is shared by NDF, which implies the presence of higher shares of cellulose, hemicellulose and lignin. However, the lower hemicellulose content of ESHW of 75.4 g/kg DM indicates the low degradability of the wastes. During biological degradation, hemicellulose is the first portion that is being utilized by the microorganisms followed by the cellulose degradation (Varma et al. 2017). The C/N ratio of all the wastes lies between 20.2 and 25.6, thus exhibiting the suitability for AD process where the optimal range is 20–30 (Zhang et al. 2013; Alvarez et al. 2010).

Batch anaerobic digestion of extruded wastes in mono- and co-digestion

From the batch AD test, the measured specific methane yield (SMY) of all the wastes, i.e. EVMW, ESHW, 1:1(E), 1:2(E), 1:3(E), 1:4(E), and 1:5(E) is presented in Fig. 1.

Mono-digestion of EVMW and ESHW resulted in the SMY of 324.6 and 200.4 mL_N/g oDM, respectively. While during co-digestion of ESHW with EVMW from 1:1(E) to

1:5(E) proportions, the highest SMY of 354.9 mL_N/g oDM was obtained while mixing ESHW with EVMW in 1:3(E) proportions. The SMY obtained for other proportions is 280.9, 311.6, 329.6 and 327.6 mL_N/g oDM for 1:1(E), 1:2(E), 1:4(E) and 1:5(E), respectively. Thus, while compared to the calculated SMY values based on individual SMY values of ESHW and EVMW in various proportions of ESHW with EVMW, the experimental SMY in 1:3(E) showed the highest increase of 20.9% (Table 2). Further, for other proportions also, the experimental yields were found to be 7.0, 10.0, 9.8 and 7.8% higher than the calculated SMY values for 1:1(E), 1:2(E), 1:4(E) and 1:5(E) proportions, respectively.

In addition to the enhancement of the SMY, it was observed that all the co-digestion proportions reached 95% of the SMY within a time period of 14–15 days, whereas ESHW reached 95% of its SMY in 21 days. This further reveals that the co-digestion of extruded wastes not only increases the SMY but also helps to attain a steady-state at a faster rate within a short period.

The pH after AD test of all the reactors lies within 7.1–7.6 and the VFA/Alkalinity ratio ranged within 0.23–0.36 and thus VFA/Alkalinity ratio remained less than 0.4, which is the optimum for a stable AD process (Sridevi et al. 2012). The highest oDM reduction of 82.6% was obtained for 1:3(E) in concurrence with its highest SMY. The oDM reduction in other wastes, i.e. EVMW, ESHW, 1:1(E), 1:2(E), 1:4(E) and 1:5(E) is 77.2, 46.9, 66.6, 72.9, 78.1 and 77.9%, respectively.

From the estimation of energy potentials, it was observed that the highest energy potential was obtained for 1:3(E) of 11.2 MJ/kg DM in concurrence with its maximum SMY. The lowest energy potential of 6.3 MJ/kg DM was attained for ESHW due to its low SMY in correlation with its poor oDM reduction, whereas the energy potential of 10.2 MJ/Kg DM was attained for EVMW. The energy potentials of other proportions are 8.7, 9.8, 10.5

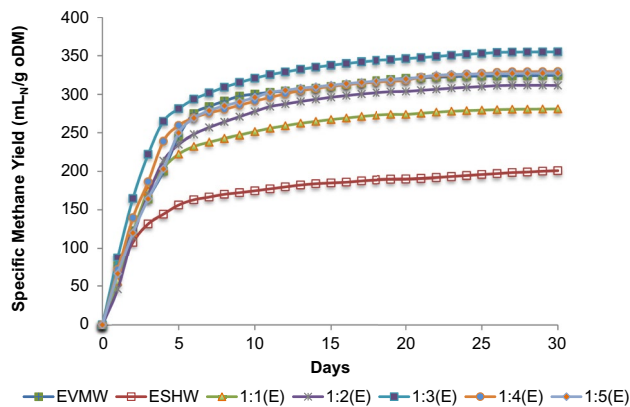


Fig. 1 Specific methane yield from batch tests

Table 2 Calculated and Experimental SMY values in different mix proportions

Proportions	ESHW ^a	EVMW ^b	Calculated SMY ^(a+b)	Experimental SMY*	% Increase
1:1(E)	100.18	162.29	262.47	280.86	7.01
1:2(E)	66.79	216.39	283.17	311.58	10.03
1:3(E)	50.09	243.44	293.53	354.92	20.92
1:4(E)	40.07	259.66	299.74	329.25	9.85
1:5(E)	33.39	270.48	303.88	327.64	7.82

^{a,b}Calculated based on individual SMY values of ESHW (200.4 mL_N/g oDM) and EVMW (324.6 mL_N/g oDM); *experimental SMY values obtained from batch tests

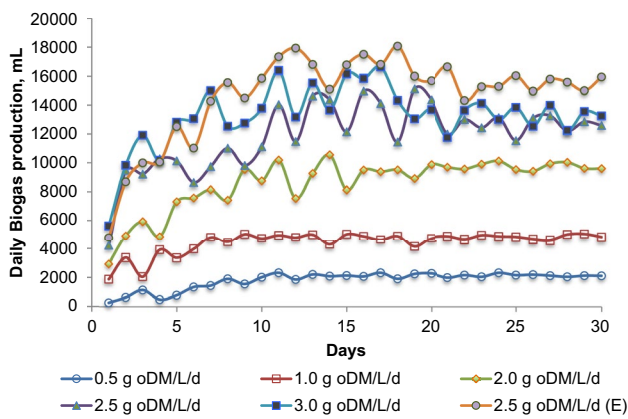


Fig. 2 Daily biogas productions at different OLR

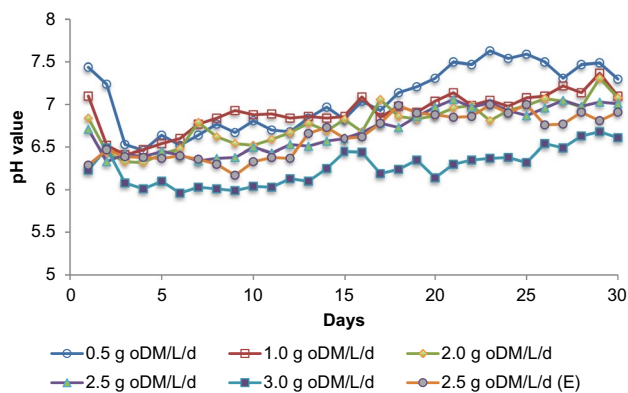


Fig. 3 Variation of Profile of pH at varying loading rates

and 10.5 MJ/kg DM for 1:1(E), 1:2(E), 1:4(E) and 1:5(E), respectively.

Continuous reactor study without extrusion pre-treatment

The continuous reactor study was carried out with co-digestion of SHW and VMW in 1:3 proportion (ESHW:EVMW) since the highest SMY was obtained at this ratio based on the results of batch AD tests. The results of continuous anaerobic reactor study, in terms of biogas production, pH, VFA, alkalinity, VFA/alkalinity ratio and ammoniacal nitrogen obtained at varying loading rates as 0.5, 1.0, 2.0, 2.5 and 3.0 g oDM/L/d are presented in Figs. 2, 3, 4a, b, 5 and 6, respectively.

At OLR of 0.5 g oDM/L/d, the average biogas production of 859.9 mL/d was attained at the beginning of the study (first week). There is a substantial difference in biogas production after the transient period (Fig. 2). Further during 21st–30th day, the average biogas production increased to 2157.9 mL/d with SBY of 431.6 mL_N/g oDM

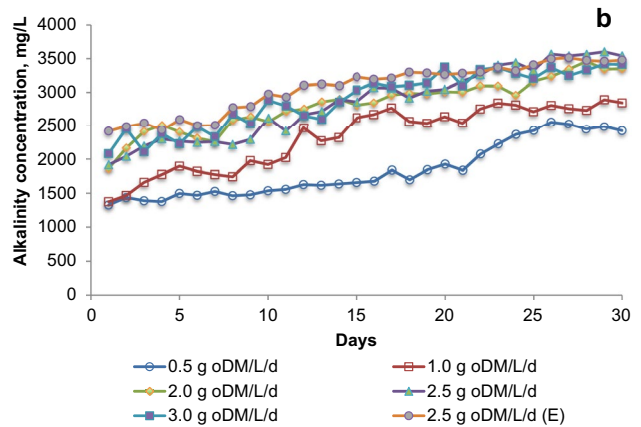
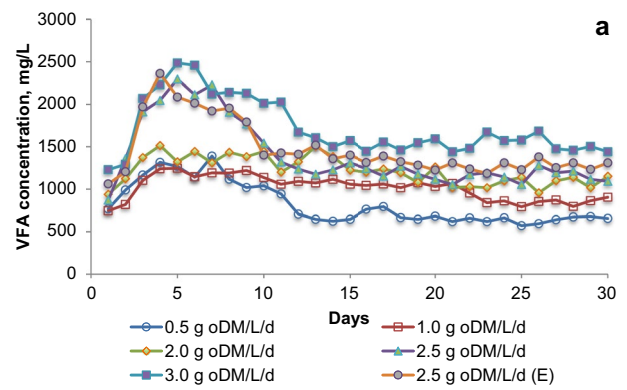


Fig. 4 Variation of Profile of VFA (a) and Alkalinity (b) concentrations at different OLRs

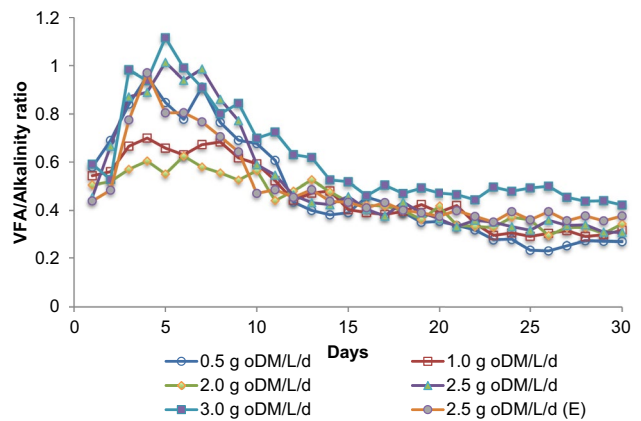


Fig. 5 VFA/Alkalinity profile at different OLR

and methane share of 65.8%. Similarly, the biogas produced during the initial week at an OLR of 1.0 g oDM/L/d was 3335.8 mL/d, which increased to 4806.9 mL/d after 20th day with SBY and methane share of 480.7 mL_N/g oDM and 62.5%, respectively. The biogas productions of 5950.8, 8810.2 and 11,197.9 mL/d were obtained at an OLR of 2.0, 2.5 and 3.0 g oDM/L/d, respectively, during

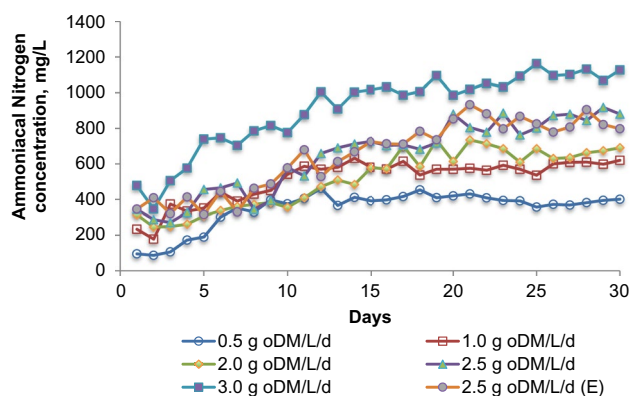


Fig. 6 Variation of Profile of Ammoniacal Nitrogen concentrations at varying OLR

the initial week. This later increased to 9715.9, 12,592.5 and 13,175.7 mL/d at OLR of 2.0, 2.5 and 3.0 g oDM/L/d, respectively. The SBY of 485.8, 503.7 and 439.2 mL_N/g oDM with methane share of 61.2%, 60.3% and 59.3% were obtained at an OLR of 2.0, 2.5 and 3.0 g oDM/L/d, respectively. The oDM reductions obtained at OLR of 0.5, 1.0, 2.0, 2.5 and 3.0 g oDM/L/d were 64.4, 68.1, 68.8, 71.5 and 65.1%, respectively. The optimal OLR was improved in the current study, during co-digestion of VMW with SHW, while compared to previously reported studies during mono-digestion of similar wastes. For example, Ganesh et al. (2014) found the optimal OLR as 2.0 g oDM/L/d during AD of fruit and vegetable market wastes.

While comparing the biogas production profiles (Fig. 2) at different OLR, it could be seen an abrupt rise and fall in the biogas production during the initial weeks; however it reached steady-state phase in almost 20 days at all OLRs. This could be due to the sudden increase in the loading, which elevates the VFA formation (Fig. 4a), and subsequent variations in the biogas production.

Further, it could be observed that at a relatively low OLR, the methane share was higher and vice versa. At higher loading, the methane share declined gradually from 65.8% (0.5 g oDM/L/d) to 59.6% (3.0 g oDM/L/d). However, the oDM removal efficiency was higher up to OLR of 2.5 g oDM/L/d (71.5%), whereas a further increase in OLR to 3.0 g oDM/L/d resulted in the decrease in oDM removal efficiency to 65.1%. This indicates that the reactor is under unstable condition normally at this higher OLR of 3.0 g oDM/L/d, which could be revealed from the drop in the oDM reduction and biogas production also. Thus, among the studied OLR from 0.5 to 3.0 g oDM/L/d, the highest SBY of 503.7 mL_N/g oDM with oDM reduction of 71.5% was attained at an OLR of 2.5 g oDM/L/d. The optimal OLR, obtained from the current study, lies

in the range of previously reported studies in the range of 1.3–3.5 g oDM/L/d (Nasiruddin et al. 2020; Ganesh et al. 2014).

Continuous reactor study with extrusion pre-treatment

The continuous reactor study of extruded wastes at 1:3(E) proportion was carried out at an optimal loading rate of 2.5 g oDM/L/d (E), obtained from the results of continuous reactor study carried out at varying loading rates without extrusion pre-treatment.

The biogas production at the beginning was observed to be 10,184.0 mL/d, which subsequently increased to 15,462.4 mL/d (Fig. 2). The highest SBY of 618.5 mL_N/g oDM, with methane share of 62.2% and oDM reduction of 82.0%, was obtained for anaerobic co-digestion of 1 proportion of extruded ESHW with 3 proportion of extruded EVMW at an OLR of 2.5 g oDM/L/d (E) when compared to non-extruded wastes. Hence, in the present study, the extrusion pre-treatment showed an increase in the SBY from 503.7 to 618.5 mL_N/g oDM with a rise in the oDM drop from 71.5 to 82.0%. The enhanced oDM reduction reveals that the extrusion as a pre-treatment prior to the AD process enhances the rate of biological degradation of biomass as a result of the structural disruption caused by the pre-treatment. Similarly, an increase in the methane yield (up to 72%) was reported in existing studies as a result of extrusion pretreatment of biomass (Chen et al. 2014; Hjorth et al. 2011). The lesser increase in the biogas yield (from 503.7 to 618.5 mL_N/g oDM) in this study compared to the reported studies on extrusion could be due to fact that the previous studies on extrusion were mainly reported for energy crops (maize silage, rice straw, etc.). The reported energy crops were found to have high DM and oDM content for which the extrusion pre-treatment showed more positive effect than the substrates with less oDM and DM content like wholesale vegetable market wastes and slaughterhouse wastes. On the other hand, it could be stated that the higher the DM content of wastes, the lower is the heating capacity which in turn consumes less energy and thus results in the increase in the temperature of the output biomass from the extruder (Hjorth et al. 2011). However, the results are highly comparable with the SBY obtained by Novarino and Zanetti (2012) as 600 mL_N/g oDM during AD of extruded organic fractions (mixed) of MSW. Further, the results of the SBY in this study are higher than the previously reported SBY, from studies on AD of VMW and SHW wastes in the range of 430–591 mL_N/g oDM (Nkemka et al. 2015; Kafle et al. 2014; Babae and Shayegan 2011; Velmurugan and Ramanujam 2011; Sridevi et al. 2012). This reveals the

positive effect of extrusion pre-treatment on increasing the energy yields during the AD process.

Energy potential of wastes during continuous reactor study

From the estimation of energy potential at varying organic loading rates from 21st day to 30th day in a continuous reactor study, the highest energy potential of 12.2 MJ/kg DM (average values) was attained at an OLR of 2.5 g oDM/L/d (E) when compared to non-extruded wastes. Among the non-extruded wastes, at varying organic loading rates, the highest energy potential was obtained at 2.5 g oDM/L/d of about 10.2 MJ/kg DM, followed by 2.0 g oDM/L/d with 10.1 MJ/kg DM. The energy potential at an OLR of 0.5 g oDM/L/d and 1.0 g oDM/L/d was 9.5 and 9.9 MJ/kg DM, respectively. The lowest energy potential of 8.7 MJ/kg DM was attained at an OLR of 3.0 g oDM/L/d. In addition to the decreased biogas production, this decrease in the energy potential could also be attributed to the decreasing trend of methane share with an increase in the loading rates.

Process performance at varying loading rates

The various process parameters, during AD process, such as pH, VFA, Alkalinity, VFA/Alkalinity ratio and ammoniacal nitrogen, play an important role in determining the reasons for the inhibitory effect on methanogens, decrease in the biogas production and reactor stability (Liu et al. 2017).

At varying loading rates, there is a drop in the pH during initial stages of each loading due to the slight accumulation of VFA followed by a subsequent increase at the end of the monitoring period, i.e. from 21st to 30th day (Fig. 3). The pH, after 21st day, of the reactor remains between 7.02 and 7.48 up to OLR of 2.0 g oDM/L/d, whereas there is a decrease in the pH to 6.99, 6.47 and 6.88 at OLR of 2.5, 3.0 g oDM/L/d and 2.5 g oDM/L/d (E), respectively. Thus, the pH at an OLR of 3.0 g oDM/L/d remained below 6.5, which indicates the instability of the reactor. This drop in the pH resulted in the drop of the SBY and oDM reduction at this loading rate of 3.0 g oDM/L/d.

From the VFA concentration profile at different OLR (Fig. 4a), it could be seen that there is a steep increase in the VFA production during the initial days (up to almost 10 days) followed by the steady-state condition observed for all loading rates. This could be visualized from the drop in the pH value during the initial stages due to VFA accumulation. However, there is a drop in the VFA concentration at the later stages due to the subsequent utilization by methanogens. Previous studies signified a VFA concentration ranging below the range of 1000–1800 mg/L for a stable AD process (Patinvoh et al. 2018; Shi et al. 2017).

The VFA concentrations of 638.1, 880.7, 1066.6 and 1151.4 mg/L were observed at an OLR of 0.5, 1.0, 2.0 and 2.5 g oDM/L/d, respectively, which does not affect the digester stability. Nevertheless, the reactor stability was declined when the OLR was increased to 3.0 g oDM/L/d with a VFA concentration of 1531.2 mg/L. This was further revealed with the decline in the SBY and oDM reduction at an OLR of 3.0 g oDM/L/d. Similarly, the decrease in the biogas production and oDM reduction as a result of VFA accumulation was observed by Chen et al. (2015) and Yadavika et al. (2004). Further, it is pertained to observe that VFA inhibitions varied up to a high concentration range of 3500–6900 mg/L during AD of VMW alone (Calabrò et al. 2019; Xu et al. 2014; Cuetos et al. 2010) and even low VFA concentration of 220 mg/L was reported during AD of ruminal contents of SHW (Nkemka et al. 2015). However, the current study overcomes both of these inhibitions by anaerobic co-digestion of SHW with VMW that in turn balances the intermediate metabolite formations during microbial metabolism within the AD system (Chen et al. 2015).

The VFA concentration of 1275.6 mg/L was observed for extruded wastes at an OLR of 2.5 g oDM/L/d (E), which did not affect the digester stability that could be seen from the maximum biogas production (15,462.4 mL/d with SBY of 618.5 mL_N/g oDM) observed. Further, this slight higher VFA concentration (1275.6 mg/L) for extruded wastes compared to the non-extruded wastes at the same loading rate with lesser VFA concentration (1151.4 mg/L), which indicates an enhanced rate of hydrolysis as a result of extrusion pre-treatment.

The alkalinity concentrations at different OLR of 0.5, 1.0, 2.0, 2.5 and 3.0 g oDM/L/d were 2336.3, 2768.9, 3204.2, 3441.4 and 3306.6 mg/L, respectively (Fig. 4b). The alkalinity concentration at extruded OLR of 2.5 g oDM/L/d (E) was 3409.9 mg/L. Hence, the alkalinity concentrations at all OLR remained in the range of 2336.3–3441.4 mg/L, which is in the optimal range for AD.

While comparing the profile of VFA and alkalinity concentration at different OLR, it could be seen that both of these parameters are contradictory to each other with their subsequent reflection in the increase or decrease in pH. The increase in the alkalinity could be attributed to the rise in the pH, thereby maintaining a sufficient buffer within the AD system, which would favour the performance of the methanogens. The variations in the VFA and alkalinity could be attributed to the synergistic effects of various parameters such as bicarbonate, CO₂, VFA and ammoniacal nitrogen within the AD system (Chen et al. 2015).

It is considered that the measurement of VFA/Alkalinity ratio during the AD process helps to predict process failures and this ratio is considered to be an indicator of reactor stability (Cuetos et al. 2010). In this study, the VFA/Alkalinity ratio was found to be 0.28, 0.32, 0.33, 0.33 and

0.46 at varying OLR of 0.5, 1.0, 2.0, 2.5 and 3.0 g oDM/L/d, respectively (Fig. 5). Thus, the VFA/Alkalinity ratio at 3.0 g oDM/L/d exceeds the optimal value for AD process (remained > 0.4), indicating the process inhibitions. This consequently resulted in the drop in the SBY and pH. At an OLR of 2.5 g oDM/L/d (E), there is an increase in the VFA/alkalinity ratio to 0.37 when compared to non-extruded waste at the same loading rate. This could be due to the slight increase in the VFA concentration (1275.6 mg/L) of extruded wastes compared to non-extruded wastes (1151.4 mg/L). This could also be seen from the slight drop in the pH (from 6.9 to 6.8) at an OLR of 2.5 g oDM/L/d (E) compared to OLR of 2.5 g oDM/L/d.

The ammoniacal nitrogen, an intermediate metabolite during the AD process, is one of the important indicators for determining the reactor stability. The ammoniacal nitrogen is released during protein and nitrogen compound degradation, which is consumed partly for the microbial metabolism and the rest being utilized for acid–base balances within the AD system (Jun et al. 2009; Zhu et al. 2007).

The ammoniacal nitrogen concentration at OLR of 0.5, 1.0, 2.0, 2.5 and 3.0 g oDM/L/d was observed to be 390.1, 587.4, 672.1, 842.8 and 1088.2 mg/L, respectively (Fig. 6), whereas the ammoniacal nitrogen for extruded OLR of 2.5 g oDM/L/d (E) remains as 841.6 mg/L. The concentration of ammoniacal nitrogen exceeding the range of 1000–1500 mg/L is inhibitory to the AD process (Chen et al. 2008; Hegde and Trabold 2019). Thus, in the present study, the ammoniacal nitrogen remains below 1000 mg/L at all OLR except at 3.0 g oDM/L/d where it is above the inhibitory range.

Comparison of effect of extrusion at optimal OLR

While comparing the results of continuous reactor study carried out with extruded and non-extruded wastes at optimal mixing ratio (1:3(E)), it was observed that the

biogas production from extruded wastes was higher (15,461.4 mL/d) at an OLR of 2.5 g oDM/L/d (E) in concurrence with its maximum oDM reduction (82.0%) compared to non-extruded wastes at the similar loading rate that showed biogas production of 12,592.5 mL/d. Further, the highest SBY and energy potential of 618.5 mL_N/g oDM and 12.2 MJ/kg DM, respectively, was obtained for extruded wastes at an OLR of 2.5 g oDM/L/d (E) compared to non-extruded wastes, which showed SBY and energy potential of 503.7 mL_N/g oDM and 10.2 MJ/kg DM, respectively (Table 3). The results reveal that the extrusion pre-treatment accelerated the hydrolysis of wastes, which would be a proficient option for enhancing the energy yield from the biogas plants.

In addition to the enhancement of biogas production from extruded wastes, the reactor stability parameters such as pH, VFA/alkalinity ratio and ammoniacal nitrogen concentration are found to be within the optimal for ensuring a stable AD process. Thus, the results showed that extrusion as a pre-treatment prior to anaerobic co-digestion process could improve the biogas production and helps in better substrate management for AD system.

Comparison of current study with existing studies on AD of extruded wastes

The results of the present study were compared with the existing reported studies on extrusion pretreatment and its impact on biogas/methane yields (Table 4). Table 4 illustrates that the extrusion pretreatment of wastes was majorly carried out on the energy crops/agro-wastes for enhanced biogas yield.

The experimental conditions and parameters of the aforementioned literature and the wastes taken for study are different from the present study, so the comparison of current results with the literature value might not provide the exact comparison. However, the current study results

Table 3 Summary of results of continuous reactor study at varying organic loading rates

Parameters	OLR (g oDM/L/d)*					
	0.5	1.0	2.0	2.5	3.0	2.5 (E)
Daily biogas production (mL/d)	2158	4807	9716	12,592	13,176	15,462
oDM removal (%)	64.38	68.09	68.79	71.48	65.07	82.01
Specific biogas yield (mL _N /g oDM)	431.58	480.69	485.80	503.70	439.19	618.49
Methane share (%)	65.83	62.46	61.17	60.33	59.27	62.18
pH	7.48	7.12	7.02	6.99	6.47	6.88
VFA (mg/L)	638.10	880.71	1066.64	1151.36	1531.21	1275.65
Alkalinity (mg/L)	2336.34	2768.99	3204.16	3441.40	3306.65	3409.91
VFA/alkalinity ratio	0.28	0.32	0.33	0.33	0.46	0.37
Ammoniacal nitrogen (mg/L)	390.14	587.44	672.14	842.82	1088.18	841.55

*All the data in this table are the average of the values obtained from 21st to 30th day, after steady state, during continuous reactor study

Table 4 Comparison of current study with existing studies on AD of extruded wastes

S.no	Feedstock	Pretreatment & process conditions	Specific biogas ^a / methane yield ^b (mL/g oDM)	References
1	Mixed vegetable, fruit and flower market wastes	Extrusion; Batch AD tests; Effective reactor volume: 400 mL; Inoculum–substrate ratio: 2; Temperature: 37 °C; Residence Time: 30 days	324.6 ^b	This study
	Slaughterhouse wastes	”	200.4 ^b	This study
	Mixed VMW:SHW (3:1)	”	354.9 ^b	This study
	Mixed VMW:SHW (3:1)	Extrusion; Continuous AD tests; Effective reactor volume: 10 L; Temperature: 37 °C; OLR: 2.5 g oDM/L/d; Residence Time: 30 days	618.5 ^a	This study
2	Vegetable market wastes	Extrusion; Batch AD tests; Effective reactor volume: 400 mL; Inoculum–substrate ratio: 2; Temperature: 37 °C; Residence Time: 30 days	319.6 ^b	Mozhiarasi et al. (2020)
	Fruit market wastes	”	332.7 ^b	Mozhiarasi et al. (2020)
	Flower market wastes	”	188.1 ^b	Mozhiarasi et al. (2020)
3	Organic fractions of MSW	Extrusion; Pilot AD plant; Digester volume: 1000 mL with 800 mL effective volume; Inoculum–substrate ratio: 0.4; Temperature: 40 °C; Residence Time: 30 days at varying OLR; OLR: 4.3 g oDM/L/d;	800 ^a	Novarino and Zanetti, (2012)
4	Rice straw	Extrusion; Batch AD tests; Effective reactor volume: 400 mL; Inoculum–substrate ratio: 0.5 Temperature: 37 °C; Residence Time: 60 days	227.3 ^b	Chen et al. (2014)
5	Maize straw silage	Extrusion; Batch AD tests (VDI standards); Temperature: 39 °C; Residence Time: 27 days	302.3 ^b	Pilarski et al. (2016)
6	Straw	Extrusion; Batch AD tests; Reactor volume: 500 mL; Inoculum–substrate ratio: 1.0; Temperature: 35 °C; Residence Time: 28 days	228.4 ^b	Wahid et al. (2015)
	Deep litter	”	231.1 ^b	
7	Rice straw silage	Extrusion; Batch AD tests; Reactor volume: 2000 mL; Inoculum–substrate ratio: 2; Temperature: 40 °C; Residence Time: 60 days	279.9 ^b	Menardo et al. (2015)
	Maize silage	”	347.6 ^b	
	Triticale silage	”	321.1 ^b	
	Mixed Rice straw silage, maize and triticale silage	Extrusion; Continuous AD tests; Reactor working volume: 5500 mL; Temperature: 40 °C; Mixing speed: 4 rpm; HRT: 60 days; OLR: 2.0 g oDM/L/d	177.3 ^b	
8	Corn straw	Extrusion; Batch AD tests; Reactor Volume: 2000 mL; VDI methods; Temperature: 39 °C	254.8 ^b	Kupryaniuk et al. (2020)
9	Maize straw	Extrusion; Batch AD tests; Reactor Volume: 1800 mL; VDI methods; Temperature: 39 °C	252.5 ^b	Kozłowski et al. (2019)
10	Maize silage	Extrusion; Batch AD tests; Reactor volume: 2000 mL; Inoculum–substrate ratio: 2; Temperature: 40 °C; Residence Time: 60 days	110.2 ^b	Panepinto and Genon (2016)

showed maximal methane yield (354.9 mL_N/g oDM) under present experimental conditions in batch AD of extruded wastes (SHW with VMW in 1:3 proportion) than the literature values (Chen et al. 2014; Pilarski et al. 2016; Wahid et al. 2015; Menardo et al. 2015; Kupryaniuk et al. 2020) and also comparable with our earlier study (Mozhiarasi et al. 2020) as shown in Table 4. Similarly, the continuous reactor study of extruded waste revealed higher biogas yield (618.5 mL_N/g oDM) at an organic loading rate of 2.5 g oDM/L/d with a reactor working volume of 10 L. Furthermore, it is well understood that the extrusion pretreatment has a significant influence on devolatilizing the complex structure of waste and increased the food availability to microorganisms, which improved anaerobic degradability and biogas yield.

Conclusion

Extrusion pretreatment of wastes (VMW and SHW) prior to the AD process and its impact on the enhancement of methane yield during the anaerobic co-digestion process in both batch and continuous scale is successfully investigated. Anaerobic co-digestion of ESHW with EVMW (1:3 proportion) showed maximal methane yield of 354.9 mL_N/g oDM in the batch AD process. Continuous AD study revealed that the extruded wastes showed an increase in the biogas yield (618.5 mL_N/g oDM) as compared to the non-extruded wastes (503.7 mL_N/g oDM) at an OLR of 2.5 g oDM/L/d. This significant enhancement in the energy production validated that the extrusion process boosted the hydrolysis rate of wastes, which improved the breakdown of biomass structure for enhanced release of organic matter. The results further reveal that extrusion pretreatment combined with anaerobic co-digestion could enhance green and clean energy generation and efficient substrate management during the AD process. Furthermore, the alternative energy production from this huge quantum of wholesale-centralized (vegetable, fruit, flower market and slaughterhouse) wastes in urban centres could provide sustainable solution for the efficient use of wastes' potency as well as reduce the quantum of wastes transported to the dumpsites/landfills and also meets the sustainable development goals. Further research on energy balance and waste conversion efficiency by increasing the dry matter content along with varying substrate combinations are recommended.

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Authors' contributions VM and SVS were responsible for conceptualization. VM was involved in methodology, investigation, data curation, original draft preparation, reviewing and editing. RB supported for performing the reaction. VM, PMBR and RB performed formal analysis. CJS, SVS and DW reviewed the results and manuscript. DW and SVS were involved in funding acquisition. SVS and RS were involved in supervision and project administration. RS was involved in project coordination.

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Declaration

Conflict of interest There are no conflicts of interest/competing interests to declare.

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