Research Article

Impact of C/N ratios and organic loading rates of paper, cardboard and tissue wastes in batch and CSTR anaerobic digestion with food waste on their biogas production and digester stability

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Abstract

The C/N ratio and organic loading rates (OLRs) are the main constraint factors of anaerobic digestion (AD) which symbols in digestion mortifications due to the rapid accumulation of volatile fatty acids (VFAs) and total ammonia nitrogen (TAN). In this study, the addition of high C-content wastes; paper waste (PW), cardboard waste (CW), and tissue waste (TW) into food waste (FW) feedstock is individually taken into consideration. At first, batch anaerobic digestion is conducted at a balanced C/N ratio of waste materials and then CSTR anaerobic digestion is carried out at different OLRs to investigate the potential of biogas production and process stability, under mesophilic conditions. In batch anaerobic digestion, co-substrate (PW + FW) feedstock is outperformed the other co-substrate feedstocks in terms of biogas production and process stability due to slow formation of VFAs and stable microbial conversion of VFAs into biogas fuel. The highest specific biogas yield (SBY) of 651 mL/gVS and specific methane yield (SMY) of 350 mL/gVS with a 57% rate of biodegradability are obtained from (PW + FW) feedstock at OLR of 15 gVS/L d. Furthermore, (PW + FW) feedstock with superb performance is then subjected to investigate the optimum OLR in CSTR digester for 30 days. The SBY of 22 L/gVS and SMY of 13 L/gVS is obtained with the utmost rate of VS reduction of 96%. The pH, alkalinity, COD, TS reduction, VS reduction, VFAs, and TAN concentration are all in the optimal range at the C/N ratio of 25 and OLR of 2 gVS/L d. This study provides new information regarding the C/N ratio and OLR for AD own great potential in improving the methane yield and productivity of PW and FW.

Keywords Anaerobic digestion · C/N ratio · Biogas · Paper waste · Tissue waste · Cardboard waste · OLRs

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1 Introduction

A huge quantity of fossil fuels is consumed for power generation in the world, which not only boosts economic burden but still also raises environmental problems. Renewable biofuel is a motivating substitute to meet the energy supplies of the world without any economic and environmental influences [1]. Continuously increasing cost and demand for waste disposal and progressively rising public concerns with the degradation of environmental quality, the energetic usage of municipal solid waste (MSW) to bioenergy has become an economical practice [2]. MSW for example, agriculture waste (AW), PW, CW, TW, and FW having C-content reveals the advantage to produce biogas [3]. According to the food and agriculture organization (FAO) of the united nations report, the annual generation of PW, CW, TW, and FW materials has been nearly 400,000 metric tons since 2010 [4]. About 1.3 billion tons of FW is wasted annually and this amount is expected to increase by 44% due to economic and population growth in the coming decades [4, 5]. In Europe, the amount of FW has been increased from 89 million tons in 2006 to 126 million tons in 2020 [6]. Due to biodegradable organic features of FW and approach to serving as bio-CH_4 sources, several studies are focused on anaerobic digestion (AD) of FW. AD process is expressed as an environmental-friendly and efficient technique for the treatment of FW and its valorization in the usage of different products, likely bio-CH_4, bio-H_2, alcohol, and VFAs [7].

AD process is considered as promising technology but has some operational limits due to waste materials characteristics, anaerobic digester design, and process conditions that can influence the performance of AD. C/N ratio of feedstock and OLR of the digester are important process conditions of AD because they indicate the process performance and digester stability [8]. The unbalance C/N ratio of feedstock can enhance the rate of formation of VFAs and TAN that are intermediate products of the AD process and they gradually accumulate in digester lead to decrease process efficiency [9]. High accumulation of VFAs and TAN in digester depends on the substrate to be used that can inhibit methanogenic activity [10]. AD using a mono-substrate of a certain variety of biodegradable waste material is facing many technical, economic, and social challenges. Particularly, AD of FW is a complicated process and reveals system instability. The rapid conversion of digestible FW to VFAs yields a drastic drop in pH at the early stage of the AD process and shows to unproductive AD performance. Moreover, the high contents of lipid and protein in FW also easily lead to inhibitory levels of TAN and long-chain VFAs [11]. The unnecessary formation of VFAs and TAN can be avoided by increasing the C/N ratio of waste materials because the anaerobic microbial populations consume carbon 25–30 times faster than nitrogen [12]. Thus, a single valorization of FW is not a desirable option compared to combining AD with other C-content rich MSW. Waste materials with high C-content can be mixed with FW to sustain the desire C/N ratio that can provide stable pH and improve methanogenic movement due to the optimal production of VFA_S and TAN. Therefore, these issues related to the AD of FW can be overcome by introducing another appropriate waste substrate in co-digestion [13].

Co-digestion of FW could be beneficial due to their balanced nutrients, less toxic chemicals, and the synergistic effect of both microorganisms [11]. This co-digestion process is an economically achievable approach and enhances pH buffering capacity as well as the bio-CH_4 yield of digester [14, 15]. However, it is still in an immature state and has a high demand to explore and develop this process. To evaluate the effect of substrate on co-digestion of FW and improve this process, many researchers have used different waste substrates, e.g., dairy manure [16], piggery wastewater [17], sewage sludge [18], thickened WAS [19], straw [20], rice husks [21], maize husks [22] and algae biomass residue [23]. Concerning most of the research in the co-digestion of FW reported up to date, more attention has been paid to the identification of new potential substrates and optimization of operational conditions. However, methane yield is still not ideal and co-digestion suffers from the digester stability and high cost of digester construction and substrate transportation. Moreover, the current knowledge of operating conditions such as C/N ratio, inoculation ratio, OLR, temperature, and time in the co-digestion of FW is not enough and affects the understanding and precise control of the digestion process.

PW, CW, and TW are other principal categories of MSW and attract great interest in the field of AD because of their environmental and economic benefits [3]. It has been reported that developed nations produce the largest portion (31%) of these waste materials and only 5–14% portion of these by developing nations [24]. These wastes are typically invented by mechanical and chemical treated wood through multiple industrial processing steps. PW, CW, and TW are thought to be promising waste substrates in the co-digestion of FW because these biodegradable waste materials are composed of lignin, cellulose, and hemicellulose which are a rich source of C-content. AD of PW, CW, and TW with FW can enhance the overall process performance and can also decrease the accumulation of AD inhibitory intermediate products by providing stable microbial conversion into biogas fuel. To the best of our knowledge, AD of PW, CW, and TW with FW has been less focused on and impact of C/N ratios and

OLRs of co-substrate feedstocks on biogas production and digester stability in batch and CSTR digester have not been investigated so far.

In the present study, an investigation is carried out to evaluate the feasibility of AD of high C-content waste materials, i.e., PW (C/N = 379), CW (C/N = 355) and TW (C/N = 188) with low C-content waster material, i.e., FW (C/N = 18) using batch and CSTR system. Additionally, the influences of operational conditions such as C/N ratios and OLRs on the process performance and stability of batch and CSTR digester are studied. The process performance and digester stability are investigated in terms of biogas and CH_4 yield, TAN, VFA, chemical oxygen demand (COD), pH, total solids (TS), and VS reduction. Moreover, an experiment was performed to assess the influence of OLR (gVS/L d) on biogas production as well as anaerobic biodegradability (D_{deg}) of co-substrate feedstocks. This study will provide sufficient information for successful anaerobic co-digestion of MSW likely PW, CW, TW with FW regarding better anaerobic digestion process and operational conditions.

2 Materials and methods

2.1 Materials

FW was obtained from a restaurant. Bones were separated and the remaining FW was grinded with a blender (Model SS3300). The grinded FW was stored at (− 20 ± 1 °C) for further use. PW, CW, and TW from a solid waste treatment plant near BUCT, Beijing, China, were collected where domestic waste material is mechanically seized. The inoculum was collected from the biogas plant in Shunyi, Beijing, China, and stored at 4 °C. The chemical properties of inoculum, PW, CW, TW, and FW are shown in Table S1.

2.2 Experimental setup and conditions for batch digestion

In batch digestion, the PW, CW, TW were mixed separately with FW on VS basis in a proportion of 1:6.51, 1:6.07 and 1:7.15 to succeed the co-substrate (PW + FW), (CW + FW) and (TW + FW) feedstocks with C/N ratio of 25, respectively. The experiment was conducted in batch digesters with a total volume of 1 L and a working volume of 0.8 L. Figure 1 illustrates the experimental setup for batch digestion. The digesters were positioned in an incubator to maintain the desired temperature (35 ± 2 °C). All the digesters were loaded with the particular quantities of co-substrates allowing to OLR of 15 gVS/L. The inoculum and substrate were adopted in line with reported in the literature [25], and the substrate/inoculum (S/I) ratio was 1:1. The digesters were loaded up with water to fill the remaining volume. The digesters were attached to the water displacement method for daily biogas volume calculations. To avoid leakage, the digesters were tightly sealed after feedings with airtight rubber corks. The digesters were finally purged by N_2 gas for 3–5 min to preserve anaerobic digestion conditions. Mixing was provided to digesters through shaking for 1 min, twice per day till the end of the digestion period. The C/N ratios for all co-substrates were calculated by using Eq. (1). The compositions

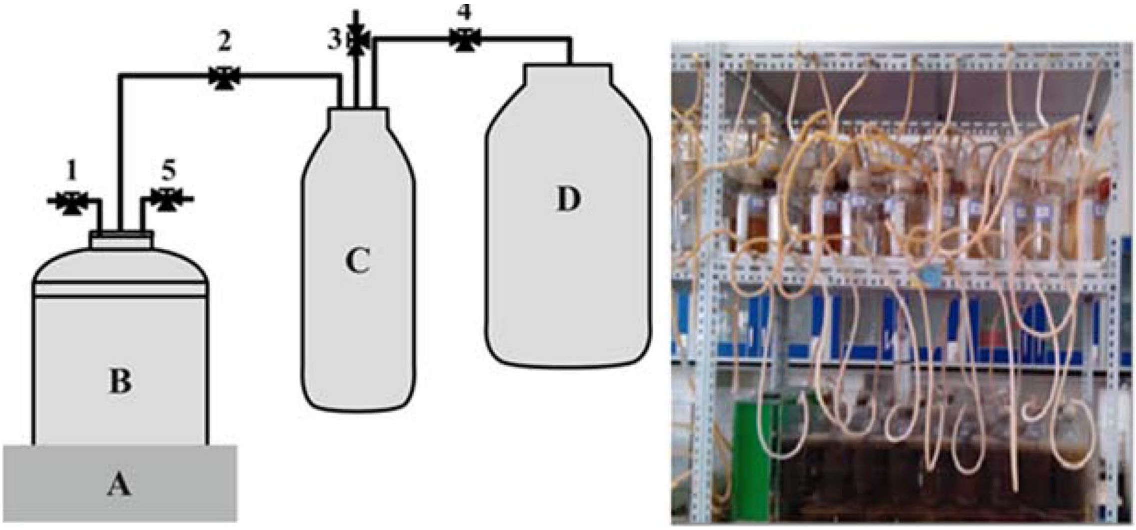

Fig. 1 Experimental setup for batch digestion. A: incubator, B: anaerobic digester, C: water displacement technique, D: water storage, 1: feed inlet valve, 2: gas flow valve, 3: gas discharge valve, 4: water control valve, 5: outlet valve

of co-substrate feedstocks for batch digestion are shown in Table S2.

$$\frac{C}{N}=\frac{\mathrm{FW}(\mathrm{TS}\times\mathrm{TOC})+\mathrm{PW}(\mathrm{TS}\times\mathrm{TOC})}{\mathrm{FW}(\mathrm{TS}\times\mathrm{TON})+\mathrm{PW}(\mathrm{TS}\times\mathrm{TON})} \quad (1)$$

where TS is total solid contents, FW is the weight of wet food waste, PW is the weight of paper waste, TON is total organic nitrogen-based on dry mater %, TOC is total organic carbon based on dry mater %.

2.3 Experimental setup and conditions for CSTR digestion

The co-substrate (PW + FW) feedstock revealed the highest SBY and SMY yield from the batch digestion process, was selected for the CSTR digestion process to explore the influence of the variation in the OLRs (2–5 gVS/L d), on biogas production and CSTR digester stability. Figure 2 shows the experimental setup for the CSTR digestion process. The CSTR digester was fixed with a top plate that supported the stirrer, stirring motor, influent point, and effluent point. The temperature of the digester was sustained at 35 ± 1 °C through a thermostatic water bath and mixing was performed by stirrer operated at a speed of 80 rpm for 15 min after every 120 min. The composition of co-substrate at different OLRs for CSTR digestion is shown in Table S3.

2.4 Analytical methods

In this study, TS, VS, pH, alkalinity, and TAN were calculated as per APHA Standard methods (1998). The total carbon (TC) and total nitrogen (TN) were measured by TC analyzer (Skalar Primacsslc, The Netherlands) and the total Kjeldahl nitrogen analyzer (Model KDN-2c, Shanghai) for calculating the C/N ratio. The lignin, hemicellulose, and cellulose (LHC) contents were calculated by the procedures proposed by [26]. The measured daily biogas volume was converted to a volume of a gas at standard temperature and pressure (STP) to ideal gas law, This was used to determine the volume of CH_4 based on respective CH_4 contents. The biogas compositions were analyzed on daily basis by a gas chromatograph (GC) (SP-2100, BeifenRuiLiCo., Beijing China) equipped with a molecular sieve (TDX-01) packed 2 m × 3 mm stainless-steel column and a thermal conductivity detector (TCD). The temperatures of the oven, injector port, and (TCD) was 140 and 150 °C, argon gas was used as the carrier gas flow rate of 30 ml/min. The methane production is the product of daily biogas production multiplying by daily measured CH_4 content. The daily biogas yield was measured by dividing the volume of biogas produced every day by the initial VS loaded into the digester. Cumulative biogas yield was calculated by summing the daily biogas yield at the end of the digestion process. Both (batch and CSTR digestion process) experiments data were statistically examined using a one-way ANOVA statistical tool of origin description 8.5.1.

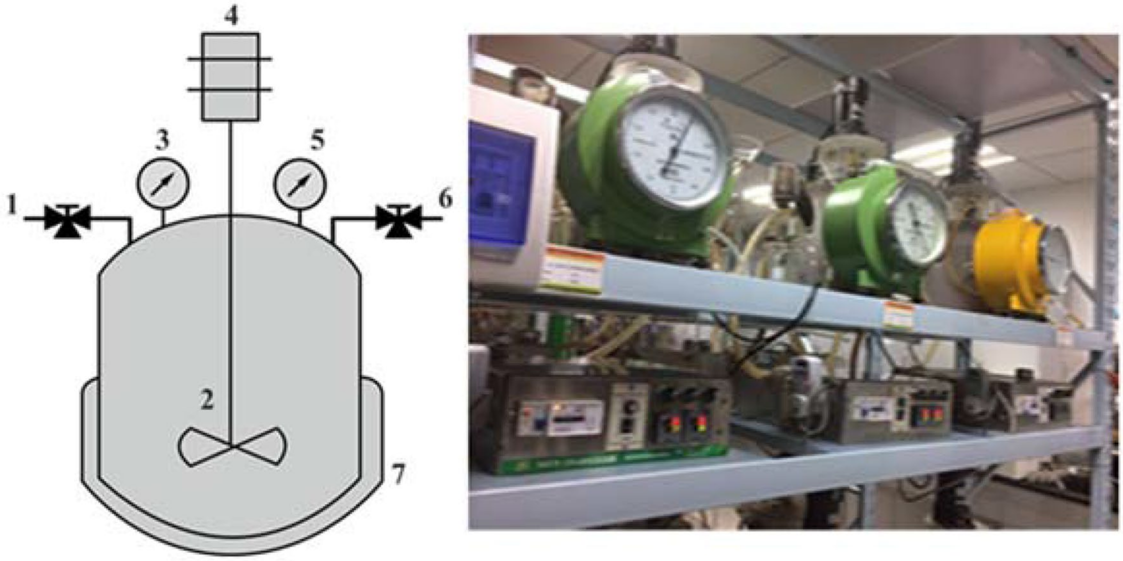

Fig. 2 Experimental setup for CSTR digester. 1: influent point, 2: mixer (stirrer), 3: temperature sensor, 4: rpm controller (stirrer motor), 5: biogas volume measuring meter, 6: effluent outlet, 7: hot water jacket

2.5 Theoretical methane yield

The empirical formula of PW, CW, TW, and FW was calculated using Eq. (2). The chemical formula of PW, CW, TW, and FW were determined by using molar mass and mass content of each element. Theoretical methane yield (TMY) of all substrate feedstocks was calculated by using Eq. (3).

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

$$\mathrm{TMY} = 100 \times 22.4 \times \left(\frac{\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}}{12n + a + 16b + 14c}\right) \quad (3)$$

2.6 Kinetic model

A kinetic model modified from the Gompertz growth equation [27] and [28] was used to describe the cumulative methane yield as follows:

$$\mathrm{BG} = \mathrm{BGP}\ \exp\left\{-\exp\left[\frac{\mathrm{R}_m e}{\mathrm{BGP}}(\lambda - t)+\right]\right\} \quad (4)$$

where BG and BGP are the cumulative methane yield (ml g VS $^{-1}$) and methane yield potential (ml g VS $^{-1}$), R_m is the maximal daily methane potential (ml g VS $^{-1}$ d), t is digestion time, λ is time lag for bacterial growth and e is the mathematical constant, i.e., 2.72.

3 Results and discussion

In the batch digestion process, the effect of co-substrate (PW + FW), (CW + FW), and (TW + FW) feedstocks with a balanced C/N ratio 25 on process performance and batch digester stability was investigated. Process performance was calculated in terms of organic pollutants COD removal, daily biogas production, and TS, VS removal efficiency, whereas digester stability was calculated in terms of pH, TAN, VFAs, and VFA/alkalinity ratio. In the CSTR digestion process, the effect of different OLRs on process performance and CSTR digester stability was examined in terms of methane yield, VS reduction, TS reduction, pH, and accumulation of VFA and TAN due to increasing OLRs. The experimental results of both processes have been described in detail in the following sections.

3.1 Effect of co-substrate feedstocks on the digestion process

3.1.1 Effect of co-substrate feedstocks on batch digester performance

The daily biogas production and methane contents of co-substrate (PW + FW), (CW + FW), and (TW + FW) feedstocks, and control mono-substrate PW, CW, and TW feedstocks, are shown in Fig. 3a, b, respectively. It was observed that co-substrates had higher daily biogas production than the mono-substrates at the initiated digestion time. The

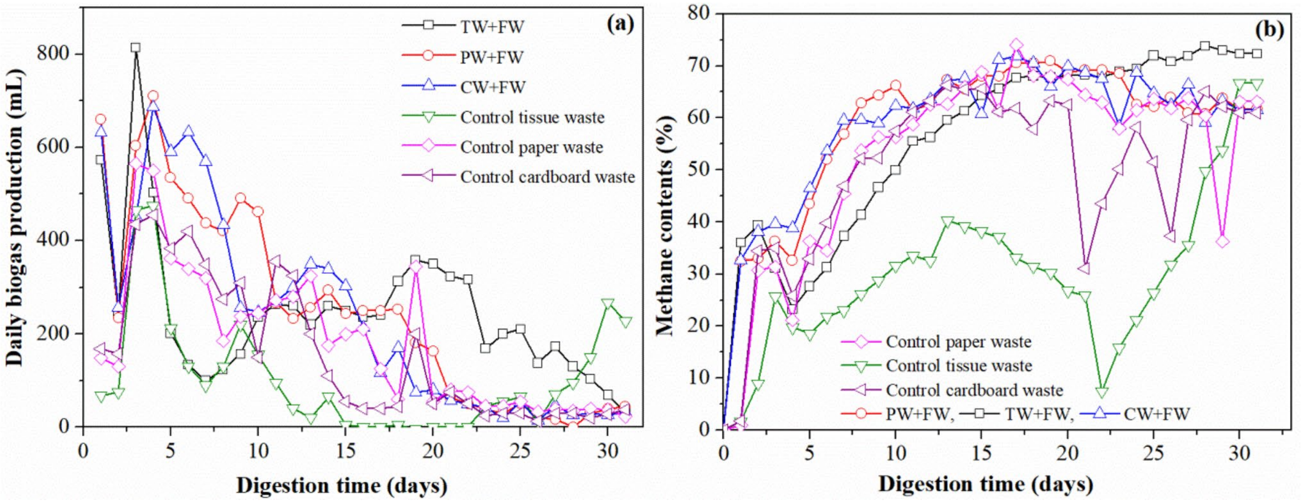

Fig. 3 Daily biogas production (**a**) and methane contents (**b**) for co-substrate (PW + FW), (TW + FW), and (CW + FW) feedstocks

addition of FW into PW, CW, and TW to balance the C/N ratio was feasible to achieve better digester performance as well as optimum anaerobic microbial activity. In the early 5 days of the digestion process, the highest daily production of biogas was approximately 710 mL, 813 mL, and 686 mL in (PW + FW), (TW + FW) and (CW + FW) feedstocks, respectively. This was possibly affected by the faster hydrolysis of easily digestible organic matters, which primarily led to more production of CO_2 in biogas and elevated gas production volume [11]. The daily biogas production of (TW + FW), (CW + FW), PW, TW, and CW feedstocks were gradually declined in the initial days of the digestion due to the inhibition process by faster formation of VFAs and TAN. Rapidly hydrolysis and acidification of high lignin contents (TW + FW) and (CW + FW) feedstocks and high C/N ratio PW, TW, and CW feedstocks, caused accumulation of digestion intermediate products [29]. The increased concentration of VFAs and unionized TAN directed the acidification of the digestion process and high strength of TAN could fail digester performance. The inhibition process indicated the deficiency of anaerobic microbe's growth as well as a decrease in their microbial conversion activity [30].

The (TW + FW) and (CW + FW) feedstocks reproduced with higher biogas after the pH accommodated with sodium hydroxide (NaOH) [31]. A large portion of organic solid matters of (TW + FW) and (CW + FW) feedstocks was converted into biogas by methanogenic microorganisms in the last 20 days of digestion after accommodated the balanced between acidogenic and methanogenic microorganisms. The (PW + FW) feedstock obligated significantly stable trend for daily biogas production than other feedstocks, might be due to the slow formation of VFAs and stable microbial conversion of VFAs into biogas fuel [32]. About 95% of daily biogas was achieved from (PW + FW) feedstock in the earlier 20 days of the digestion and the rest of that gas was achieved in the last 10 days. In the AD of PW, CW, and TW feedstocks, the results showed that inhibition of the digestion process by the accumulation of TAN occurred because of multi-times pH adjustment with NaOH. TW feedstock required a long time for organic matter degradations, as compared to other co-substrates and mono-substrates. However, TW feedstock restarted to produce biogas after 25 days of digestion, could be attributed to suitable adjustment of pH which provided fast conversion of TW to biogas by methanogenic microorganisms.

Similarly, methane contents for all co-substrate feedstocks were above 40% in the first 5 days of digestion. However, the mono-substrates showed lower methane contents compared to co-substrates. For co-substrate feedstocks, the overall methane content was found to be 70–75% during the whole digestion. An increase of 20–40% was observed in the methane contents in the early digestion period. After 10 days of digestion, methane contents reached in the range of 50–70% which revealed high digester biodegradability and better digester performance. The (PW + FW), (CW + FW), and (TW + FW) feedstocks offered the advantages of balance of nutrients, C/N ratios equilibrium, and increased buffering capacity of the AD process [32]. Therefore, the maximal potential of methane content was obtained in the earlier 20 days of digestion time. Whereas, the maximal potential of methane contents for CW and TW feedstocks was obtained in the last few days of the digestion period. The methane content of TW feedstock increased from 2 to 25% in the first 20 days of digestion and then rapidly increased in the last 10 days. Meanwhile, the methane content of CW feedstock also started to decline after 15 days of digestion. This probably caused by the increase of CO_2 in biogas due to the acidifying microorganisms which may result in VFAs accumulation [33]. After 20 days of digestion, the pH was re-adjusted with NaOH. The CW and TW feedstock showed slow improvement in methane contents and demanded more days for stable digestion as compared to PW feedstock. The major drawback of the mono-substrate AD process was a requirement of long digestion time that could affect the process by the deficiency of nutrients and a buffering agent for pH modification [34].

3.1.2 Effect of co-substrate feedstocks on cumulative biogas and methane production

The effect of co-substrate (PW + FW), (TW + FW), and (CW + FW) feedstocks and control mono-substrate PW, TW, and CW feedstocks on cumulative biogas and methane production are shown in Fig. 4a, b. The final effluent parameters of co-substrate feedstocks for digester performance as well as process stability are listed in Table 1. The cumulative biogas production (CBP) from (PW + FW), (TW + FW) and (CW + FW) feedstocks were 7814 mL, 7697 mL, 7370 mL and that from PW, TW, and CW feedstocks were 5637 mL, 3232 mL, and 4890 mL, respectively. In the first 20 days of digestion, about 95% of CBP was achieved from (PW + FW) feedstock and the rest of that was obtained in the last 10 days. The (PW + FW) feedstock revealed significantly higher SBY of 651 mL/gVS and that associated with (TW + FW) and (CW + FW) feedstocks were 640 and 641 mL/gVS, respectively. This indicated during the fermentation process, methanogenic phase microorganisms utilizing H_2 as electron donors and CO_2 were used to produce methane by microorganisms. Also, the digester performance was decreased by a higher concentration of VFAs and TAN of (CW + FW) and (TW + FW) in co-substrates, it revealed that the growth rate of methanogenesis microorganisms and rate of CO_2 consumption into methane production was the lower [35].

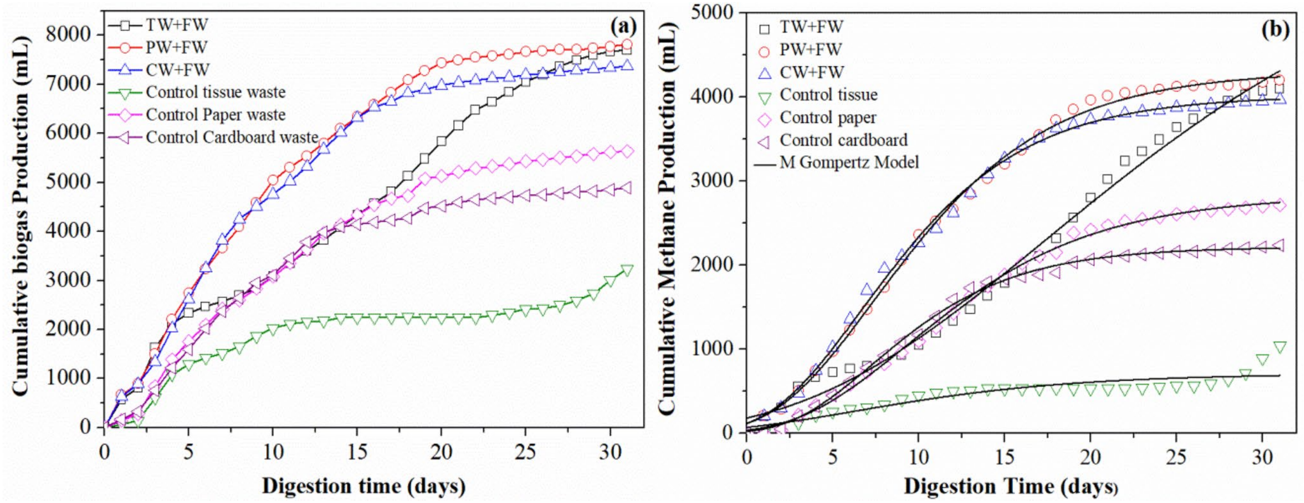

Fig. 4 Cumulative biogas production (**a**) and cumulative methane production (**b**) of co-substrate (PW + FW), (TW + FW), and (CW + FW) feedstocks

Table 1 Final effluent parameters of co-substrate feedstocks for digester performance and process stability

Parameters	Units	PW + FW	TW + FW	CW + FW
CBP	(ml)	7814	7697	7370
SBY	(mL/gVS)	651	641	614
SMY	(mL/gVS)	350	341	331
TMY	(ml)	621	615	623
Initial pH	–	7.67 ± 0.0	7.69 ± 0.0	7.62 ± 0.0
Final pH	–	7.30 ± 0.0	7.17 ± 0.0	7.24 ± 0.0
Alkalinity (TA)	(mg/L)	3650 ± 0.0	3583 ± 0.2	3367 ± 0.4
Biodegradability	(%)	57 ± 0.4	55 ± 0.0	53 ± 0.48
TAN	(mg/L)	733 ± 0.0	723 ± 0.2	681 ± 0.2
COD	(mg/L)	23,932 ± 3.0	20,247 ± 1.5	24,362 ± 1.3
VFA_S	(mg/L)	25 ± 0.0	37 ± 0.0	45 ± 0.0

CBP cumulative biogas production, *SMY* specific methane yield, *TMY* theoretical methane yield, *SBY* specific biogas yield

The cumulative methane production from (PW + FW), (TW + FW) and (CW + FW) feedstocks were 4200 mL, 4096 mL, 3972 mL, and that from PW, TW, and CW feedstocks were 2716 mL, 1046 mL, and 2240 mL, respectively. The SBY of 651, 641, and 614 mL/gVS was observed in (PW + FW), (TW + FW) and (CW + FW) feedstocks, respectively. The specific methane yield (SMY) of 350, 341, and 331 mL/gVS was observed in (PW + FW), (TW + FW) and (CW + FW) feedstocks, respectively. Significantly the high SBY and SMY were measured in (PW + FW) feedstock, which was 27% and 35% higher than unbalanced C/N ratio PW feedstock, respectively, could be attributed to high degradability of organic solids content in (PW + FW) feedstock. The biodegradability of PW feedstock increased when digested with easily degradable organic waste FW, improved overall digester performance [36]. The obtained results were compared with reported results in the literature, where biogas yield of 531 mL/gVS was achieved in the co-digestion of FW with manure [37] and biogas yield of 827 mL/gVS was obtained when MSW was digested with FW at different C/N ratios [10]. Also compared with reported study, when CW co-digested with FW and higher substrate alterations (≤ 48%) and H_2 yield (62 mL/gVS) was succeeded at low load [38]. Moreover, a close difference was observed in the results of co-substrate feedstocks. For (PW + FW) feedstock, the AD process was found in stable pH and superior methanogenic activity due to improved buffering results of the AD medium. For (TW + FW) and (CW + FW) feedstocks, the additional use of the calming agent for pH adjustment to maintain the balance between VFAs, acidogenesis and methanogenesis microorganism, represented more days require for better digestion performance. Therefore, the maximal of biogas potential was achieved for (PW + FW) feedstock in earlier short time of digestion as compared to (TW + FW) and (CW + FW) feedstocks.

3.1.3 Effect of biodegradability on batch digester performance

To evaluate the batch digester performance, the biodegradability for co-substrate (PW + FW), (CW + FW), and (TW + FW) feedstocks was calculated. Theoretical methane yield (TMY), specific methane yield (SMY), and biodegradability of organic solids for (PW + FW), (TW + FW) and (CW + FW) feedstocks are shown in Table 1. The (PW + FW) feedstock revealed the highest SMY and biodegradability as compared to the (TW + FW) and (CW + FW) feedstocks. The SMY of 350 mL/gVS and rate of biodegradability of 57% was observed for (PW + FW) feedstock. This finding showed that an effective amount of anaerobic microbes in the (PW + FW) feedstock could beneficial to increase the rate of biodegradation during anaerobic digestion [32]. As the gradual increased in the lignin contents, the methane yield decreased in (TW + FW) and (CW + FW) feedstocks due to the high accumulation of VFA that caused not only the unsterilized process but also affected the buffering capacity of the process. AD of lignocellulosic wastes such as tissue and cardboard substrate reported causing process inhibition as a result of the presence of a high portion of lignin and unbalanced nutrients [12].

3.1.4 Effect of co-substrate feedstocks on batch digester stability

The pH value, the ratio of VFA/alkalinity, and the concentration of TAN are parameters that predict the digester stability of AD. Therefore, various samples of digestate were examined to evaluate the process stability. Initial and final pH of (PW + FW), (TW + FW), and (CW + FW) feedstocks are shown in Table 1. The pH of (PW + FW), (TW + FW), and (CW + FW) feedstocks was in the range of 7.1–7.8 for digestate effluents, revealed the stable performance of digester [39]. Nevertheless, a slight variation in final pH was observed for all feedstocks as compared to the initial pH. This might be as a result of the formation of a higher amount of TAN and VFAs. The deionized ammonia could lead to enhance concentration of TAN that reflected on pH raise and the rate of biogas production could be lower [12]. The pH of digesters contained (TW + FW) and (CW + FW) feedstock was more decreased than that of the digester with (PW + FW) feedstock. This phenomenon probably caused by the accumulation of ammonia-N in the digester with feedstock of low biodegradability rate and led to high pH [40]. The pH was decreased in this study as a result of a high portion of lignin in (TW + FW) and (CW + FW) feedstocks and rapidly hydrolysis as well as acidification of these lignin contents. The hydrolysis of lignin contents restricted due to the recalcitrance of their major macromolecular components and a pH stabilizing agent was used for the neutralization of VFAs. The pH was stabilized by added 3–5 mL of (NAOH) stabilizing agent maintain pH in (AD). Moreover, Murto et al. [41] reported that FW produced ammonia as ammonium bicarbonate in the solution due to enrichment of the nitrogen contents for instance proteins and offered buffering capacity during degrading to the digesters.

The TAN production in digesters with (PW + FW), (TW + FW) and (CW + FW) feedstocks were 732 mg/L, 723 mg/L, and 681 mg/L and PW, TW and CW feedstocks were 560 mg/L, 518 mg/L, and 532 mg/L, respectively at the end of digestion period. In digester feed with co-substrate feedstocks showed high TAN concentration due to the high fraction of FW as compared to mono-substrate feedstocks. This phenomenon could be due to the easy biodegradability of PW, CW, and TW with FW which resulted in faster release of ammonia and nitrogen [11]. FW produced ammonia as ammonium bicarbonate in the solution due to the enrichment of the nitrogen contents and used as a pH stabilizing agent for the neutralization of the fatty acid [41]. TAN formation in digesters containing (TW + FW) and (CW + FW) feedstocks and mono-substrate feedstocks was more decreased than that of the digester with (PW + FW) feedstock. This phenomenon attributed to the high growth of methanogenesis microorganisms and a large amount of CO_2 consumed by methanogenesis microorganisms to produce methane in a digester with (PW + FW) feedstock [32].

Also, alkalinity and VFAs are significant parameters to examine digester stability. At end of digestion, the effluent alkalinity values in digesters with (PW + FW), (TW + FW) and (CW + FW) feedstocks were 3650 mg/L, 3583 mg/L, and 3367 mg/L, respectively. The alkalinity values were in the range of 3300–3700 mg/L. The alkalinity value closed to 4000–5000 mg/L indicated higher buffering capacity that could enhance the stability of digester [36]. The effluent alkalinity for (PW + FW) feedstock was closer to 4000 mg/L and the buffering capacity was much better as compared to (TW + FW) and (CW + FW) feedstocks. The VFAs production for all co-substrate feedstocks was in the range of 18–35 mg/L. For better process stability, VFAs must remain less than 50 mg/L and the concentration of propionate more than 1000 ppm revealed the ability to inhibit the AD process [42]. VFA concentrations found less than 50 mg/L for all co-substrate feedstocks in this study, which showed the high digester stability of the AD process. The final concentrations of alkalinity and VFAs for co-substrates were shown in Table 1.

3.2 Effect of OLRs on the digestion process

Afterward, we decided to evaluate the effect of OLRs on digester performance and process stability. It was observed that co-substrate (PW + FW) feedstock revealed higher performance compared to other studied co-substrate (TW + FW) and (FW + CW) feedstocks. Consequently, the (PW + FW) feedstock was selected to investigate the effect of OLRs on biogas production and CSTR digester stability.

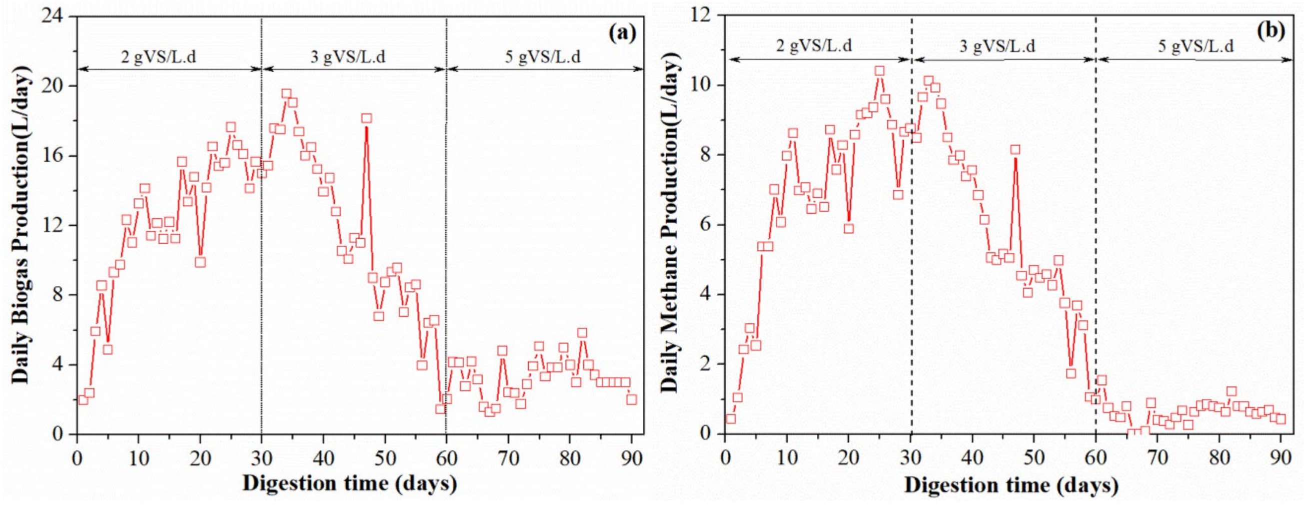

Fig. 5 Daily biogas production (**a**) and daily methane production (**b**) of co-substrate (PW + FW) feedstock at different OLRs

3.2.1 Effect of OLRs on CSTR digester performance

Effect of different OLRs on the daily biogas production as well as daily methane production is shown in Fig. 5a, b). At OLR of 2 gVS/L d, the daily biogas production continuously increased until 35 days of digestion time and then began to decline. The daily biogas production continuously decreased with the digestion time, when OLR was increased from 3 to 5 gVS/L d. This declined in the daily biogas production at high OLRs could be due to the accumulation of VFAs and the unbalance of anaerobic microbes to the organic substrate in the digester [43]. This phenomenon could be due to high OLRs having a substrate that consumed time for anaerobic microorganisms to digest organic compounds [36]. At an early stage, the acidification phenomenon was observed with OLR of 3 and 5 gVS/L d but recovered later. It ascribed to the improvement in the biodegradability of digesters [43]. More than 60% of daily biogas production was produced in the first 30 days at OLR of 2 gVS/L d and the rest of that was produced in the other 60 days with high OLRs. At low OLRs, high anaerobic bacterial action led to consuming the organic contents of the substrate effectively as compared to high OLRs with a low anaerobic bacterial action [44].

In the literature, the digestion time and biodegradation rate of organic matters reported depending on the concentration of microorganisms and their activity in digester [45]. The daily biogas production was observed to be increased in early 5 days (30 to 35 days) at OLR of 3 gVS/L d and biogas production became slower in the next days at OLR of 3 and 5 gVS/L d. It was probably due to the high rate of formation of VFAs which led to unionized ammonia-N accumulation and acidification of the medium [30]. The inhibition of biogas production took place when the pH was below 6 [46]. The alkaline solution was used to adjust pH value and biogas production resumed to normal conditions [31]. OLRs directly influenced the rate of methane production. The daily methane production gradually increased in the digestion phase for 30 days at OLR of 2 gVS/L d. Daily methane production was observed to gradually decrease for OLR of 3 gVS/L d, while low daily methane production was achieved at OLR of 5 gVS/L d. The digester having OLR of 2 gVS/L d was observed with the highest methane 61% which described the superior performance of AD.

3.2.2 Effect of OLRs on cumulative biogas and methane production

The effect of different OLRs on cumulative biogas as well as methane production is shown in Fig. 6a, b. Cumulative biogas production from the digesters having OLR of 2, 3, and 5 gVS/L d, was 362, 344, and 96 L, respectively. The cumulative biogas production at higher OLRs was declined due to the accumulation of VFAs in the digester [36]. Cumulative biogas production was 1.05 at OLR of 2 gVS/L d, and 3.77 times higher than that at OLR of 3 and 5 gVS/L d, respectively. A decrease of about 4.97% and 73.48% was observed in the cumulative biogas production when the OLR was increased 3 to 5 gVS/L d, respectively. The sudden change in OLRs which may be inhabited for microbial activity [44].

SBY was observed to be 22, 14, and 3 L/gVS, and SMY was observed to be 13, 8, and 1 L/gVS at OLR of 2, 3, and 5 gVS/L d, respectively as listed in Table 2. The OLR of 2 gVS/L d showed the highest CBY and CMY among all, while OLR of 5 gVS/L d showed the lowest yields of CBY and CMY. Surprisingly, the high rate of biodegradability

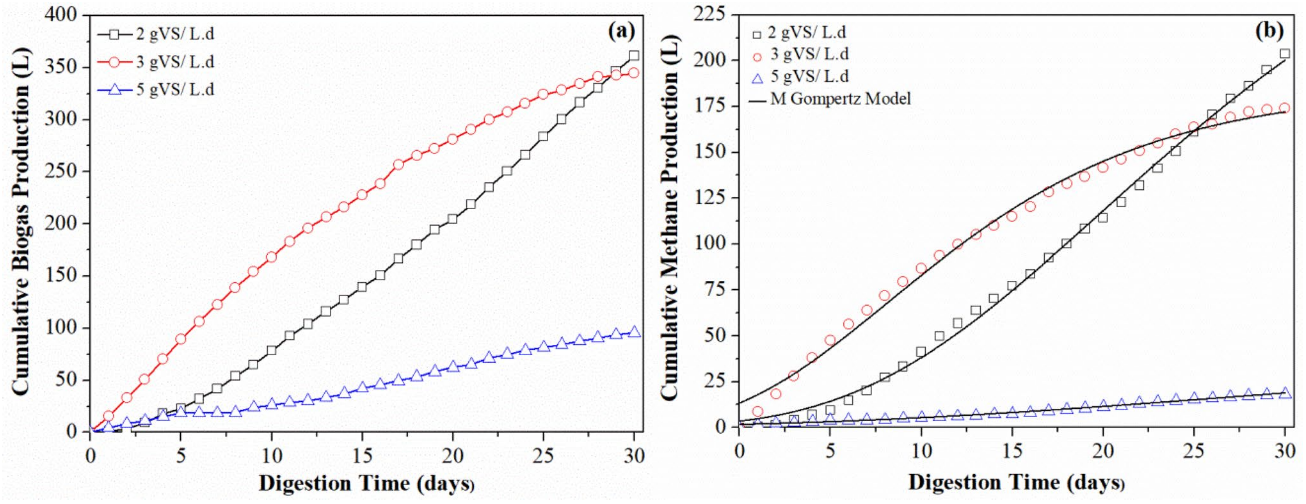

Fig. 6 Cumulative biogas production (**a**) and cumulative methane production (**b**) of co-substrate (PW + FW) feedstock at different OLRs

was detected in the digester with OLR of 2 gVS/L d, which could be the result of a high concentration of anaerobic microorganism. The decrease in biogas production could be attributed to a high rate of formation of VFAs than the rate of conversion in digesters that could be caused by unbalanced interaction of loaded organic matters and anaerobic microorganism for their microbial transfiguration [36]. The results found in this study were compared to reported work where CMY of 0.25 $m^3CH_4/kgVS$ and VS reduction of 88% at OLR of 1.4 kgVS/(m^3 d) was obtained when anaerobic digestion of vegetable waste at 1.4, 2 and 2.75 kgVS/(m^3 d) [43]. During AD of MSW, CBY was 9.3, 10.7, and 17.7 L, and CMY were 84.3, 101.0, and 168.4 mL/gVS, achieved at OLR of 5.1, 10.4 and 15.2 g/$LCOD_S$, respectively [47]. Li et al. [48] reported to the effects of (RS/CM) feedstock and OLRs on the anaerobic co-digestion of rice straw and cow manure with ratios of 0:1, 1:0, 1:1, 1:2 and 2:1 at OLR of 3.0, 3.6, 4.2, 4.8, 6.0, 8.0, and 12.0 kgVS/(m^3 d). The SBY of 383.5 L/kgVS and cumulative biogas production rate of 2.30 m^3/d was obtained at OLR of 6 kgVS/(m^3 d).

Table 2 Parameters of co-substrate (PW + FW) feedstock for digester performance and process stability at different OLRs

Parameters	Units	2 gVS/L d	3 gVS/L d	5 gVS/L d
CBY	L	362	344	96
SBY	L/g VS	22	14	3
CMY	L	204	174	18
SMY	L/g VS	13	8	1
TMY	mL	618	618	618
TS reduction	%	94 ± 0.4	87 ± 0.12	85 ± 0.35
VS reduction	%	96 ± 0.2	91 ± 0.1	82 ± 0.13
Final pH	–	6.94 ± 0.1	6.11 ± 0.0	5.0 ± 0.2
Ammonia-N	mg/L	567–364 ± 0.09	315–185 ± 0.03	217–210 ± 0.02
COD	mg/L	13,890 ± 0.97	94,780 ± 3.19	72,853 ± 2.89

CBP cumulative biogas production, *SBY* specific biogas yield, *CMY* cumulative methane yield, *SMY* specific methane yield, *TMY* theoretical methane yield

3.2.3 Effect of OLRs on TS and VS reduction

The characteristic of biomass degradation can be shown in terms of TS and VS reduction. The effect of different OLRs on the TS and VS reduction is shown in Fig. S1(a). The measuring frequency of TS and VS reduction was after every 10 days of digestion periods to evaluate the influence of OLRs on the digester performance. The highest TS and VS reduction of 96 ± 0.4% and 94 ± 0.2% was observed, respectively when digester was operated at 2 gVS/L d, and they decreased with the increase in OLRs. The results were in good agreement with bio-methane production and biogas production values when OLRs was increased to evaluate the digestion performance [44]. This phenomenon could be due to the optimum feeding proportion of (PW + FW) feedstock used in 2 gVS/L d, as compared to other ORLs, this led to higher VS conversion as reported in the literature [43]. The VS reduction rate decreased to 91% and 82% at 3 and 5 gVS/L d, which could be due to the slow degradation of high feeding lignocellulosic substrates. This led to a decline in the degradability of long-chain lignocellulosic organic compounds by lowering the activity of anaerobic microorganism as a result of the improper availability of nutrients. The TS reduction values found to be increased from 80% to 85% at the end of digestion, indicated the presence of acidic conditions in the digester which resulted in the lower conversion of TS [30].

3.2.4 Effect of OLRs on pH

The pH is a sensitive parameter that described the stability of digester during CSTR digestion. The effect of different OLRs on pH is shown in Fig. S1(b). Initially, the pH of CSTR digester was higher due to lower formation of VFAs and rate of hydrolysis at the initial digestion process but gradually declined with digestion time due to the high concentration of VFAs [33]. With the increase of OLRs from 3 to 5 gVS/L d, a drop in pH was observed from 6.5 to 5.0 which was a sign of VFAs formation. However, the enzymatic activity of the hydrolytic enzyme could cause an acidic medium, when OLRs increased from lower to higher. The methane content in the biogas was also less than 40% and biogas production was lower at high OLRs. For re-biogas production and additional buffering capacity of the digesters to used alkaline reagent to resolve the issue for a decline in pH [31].

3.2.5 Effect of OLRs on CSTR digester stability

The digestate samples were also analyzed in terms of COD, VFAs, and TAN to investigate the CSTR digester stability, after every 10 days of digestion. The effect of (PW + FW) feedstock with different OLRs on CSTR stability and digester performance is shown in Table 2. The amount of COD was analyzed to describe the biodegradability rate of digester in terms of hydrolysis and methanogenesis activity during digestion and end of digestion. At 2 gVS/L d, the organic pollutant removal efficiency was higher as compared to the other two OLRs. A lower amount of COD showed a high hydrolysis rate for biomass and total conversion of VFAs into biogas by methanogenic bacteria [47]. When OLR was changed from 2 to 5 gVS/L d, low degradability of digester was observed. At higher OLRs, the methanogenic bacteria activities could be reduced due to a higher concentration of small chain hydrocarbons and lower rates for their conversion. Therefore, the accumulation of VFAs was high and resulted in an effect on digester stability with increasing digestion retention time.

TAN concentration is a promising parameter to evaluate the significance of CSTR digester stability. At OLR of 2 gVS/L d, the deionized ammonia-N concentration was 567–364 mg/L, which was approximately suitable for the AD process. On the other hand, it enhanced the buffering capacity of the process due to stable pH and increased the formation of NH_4HCO_3 in digestion solution to overcome the rapid formation of VFAs [32]. With the increasing OLRs, the ammonia–N_2 concentration was very low due to the improper ionization of proteins. It is reported that the rapid decrease in pH because of the incomplete degradation of ammonia ions influence the digester stability and rate of biogas production [49]. In this study, the concentration of ammonia-N_2 was low according to the suggested range of ammonia for the CSTR process at higher OLRs. However, the biogas production was badly affected at lower ammonia-N concentration. A lower amount of organic matter could easily degrade by anaerobic microorganism for biogas production. But it was reported that lower anaerobic microorganism could accumulate VFAs and play a role to reduce biogas production [50].

3.3 Kinetic and statistical analysis

Gompertz growth equation was used for nonlinear regression on the cumulative methane yield as well as digestion time as shown in Figs. 4(b) and 6(b). In this study, R^2 value was observed in the range of 0.997–0.998 which reflected the feasibility of the predicted model. The maximum cumulative methane yield (BGP) and maximum daily methane yield (R_m) of feedstock were decreased with an increase in the OLRs. The results from statistical analysis also showed a significant difference ($\alpha < 0.05$) in these parameters. The experimental values of CMY were in agreement with the model prediction values of BGP. Correspondingly, the lower λ value for co-substrates (PW + FW) and 2 gVS/L d, indicted the higher rate of biodegradation with appropriate C/N balance and anaerobic microbial population. These results indicated that the increase in OLRs from 2 to 5 gVS/L d, the digester was overloaded and inhibited by the accumulation of TAN and VFAs. A similar trend was observed in the results of different co-substrates. These facts indicated that the higher ratio of lignocellulosic matters in co-substrates (CW + FW) and (TW + FW) could lead to the accumulation of VFAs resulting in low methane yield (Table S4).

4 Conclusion

Anaerobic co-digestion of PW, TW, CW, with FW, was found an effective and economical approach for the conversion of waste mass into biogas containing methane. The biogas production performance and digester stability assessment of the co-digestion of PW, TW, CW, with FW were performed at a balanced C/N ratio of 25 in the batch digester and then at OLR of 2–5 gVS/L/d in CSTR digester. The pH and total alkalinity were found to be between 7.1 and 7.7 and 3376 to 3650 mg/L, respectively, showing that the batch digestion process did not experience inhibition by acidification. Moreover, VFA and TAN were found to be below the recommended maximum limit and hence revealed that the AD was stable in the batch digester. The

(PW + FW) feedstock showed higher biogas production and digester stability than other studied co-substrate and mono-substrate feedstocks. The outperformed (PW + FW) feedstock was also investigated in CSTR digester at different OLRs. The performance of the CSTR digester was decreased with the increase in OLR resulted in low pH stability for AD and reduced methanogenic activities due to the accumulation of VFAs. For AD of (PW + FW) feedstock in CSTR digester, the optimum OLR was observed to be 2 gVS/L d. High biogas and methane production, VS and TS reduction, and ammonia–N_2 concentration was achieved at 2 gVS/L d.

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Compliance with ethical standards

Conflicts of interest There are no conflicts to declare.

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