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ORIGINAL ARTICLE

Anaerobic co-digestion of linen, sugar beet pulp, and wheat straw with cow manure: effects of mixing ratio and transient change of co-substrate

Mahmoud Elsayed[1] · **Yves Andres**[3] · **Walid Blel**[2]

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Abstract

This study concerns the improvement and sustainability of producing methane (CH_4) from the co-digestion of cow manure (CM), sugar beet pulp (SBP), linen (Ln), and wheat straw (WS). The first step involved co-digesting CM, Ln, and WS at various mixing ratios (CM/Ln/WS) in batch reactors to ascertain the best gas production. Biochemical methane potential (BMP) tests were carried out under mesophilic conditions using sludge from a wastewater treatment plant as an inoculum. The highest CH_4 production (351 mL/g VS_{add}) and volatile solids removal rate (72.87%) were observed at the mixing ratio 50/25/25 and the lowest CH_4 production (187 mL/g VS_{add}) was recorded at the ratio 25/25/50. A kinetic analysis was carried out to suggest the best strategy for methane production based on the ratio of substrates in the mix. The second step involved co-digesting CM, SBP, Ln, and WS in a semi-continuous stirred tank reactor to study the influence of a transient change in co-substrate on gas production and reactor performance. The rate of biogas production doubled with the transient change of co-substrate from WS to SBP, which may be due to the SBP being more easily biodegradable than WS.

Keywords Cow manure · Linen · Wheat straw · Sugar beet pulp · Mixing ratio · Transient change of co-substrate

1 Introduction

It is a major goal for many European Union (EU) nations to increase their production of green energy from renewable resources. The production of energy from biogas, in the form of electricity, has developed significantly in the EU as a result of its environmental and economic advantages [17]. Over the last few decades, a huge quantity of animal manure has been disposed of by traditional methods, which represents a main source of air and water pollution [20].

Anaerobic digestion (AD), where a combination of bacteria convert the organic waste to methane (CH_4) and other gases [9], is an effective treatment for manure. However, digesting manure alone results in low biogas production [6], and several authors have tested the anaerobic co-digestion of manure with other waste materials, such as agricultural waste, to enhance production (Liu, Jinming, Changhao Zeng, Na Wang, Jianfei Shi, Bo Zhang, Changyu Liu, 2021). Improvements in carbon to nitrogen (C/N) ratio, feedstock nutrient balance and gas production have been observed as a result of mixing the nitrogen-rich manure with the high carbon content of agricultural waste [12].

Of all agricultural waste materials, sugar beet pulp (SBP) appears to be a suitable substrate for AD due to its high carbohydrate content [28]. Total SBP production in the EU was 207.93 million tonnes in 2018 [15]. Wheat straw is another widely available crop worldwide, with 771.71 million tonnes produced in 2017 [14].

Crop residues from sugar beet pulp, linen (Ln) and wheat straw (WS) are some of the best co-substrates to mix with animal manure for improved CH_4 production and alkalinity, and increased bacterial activity (Elsayed et al., 2017; Yang et al., 2021).

Manure has been digested alone and in co-digestion with SBP in previous studies, but the improvement in CH4 production by adding Ln and WS to the co-digestion of manure

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✉ Mahmoud Elsayed
m.elsayed@aswu.edu.eg

[1] Civil Engineering Department, Faculty of Engineering, Aswan University, Aswan 81542, Egypt

[2] Oniris, Université de Nantes, GEPEA, CNRS UMR 6144, 44600 Saint-Nazaire, France

[3] IMT Atlantique, GEPEA, UMR CNRS 6144, Cedex 3, 4 Rue Alfred Kastler, 44307 Nantes, France

and SBP, and study of the effects of transient co-substrate changes using different waste materials (in multi-substrates) is poorly documented. Fonoll et al. [16] showed that replacing the co-substrate with a similar feedstock did not result in system failure. Fanget al. [13] reported that using SBP as a co-substrate improved CH_4 production from the anaerobic digestion of manure. Elsayed et al. [8] reported that CH_4 production from the anaerobic co-digestion of sludge and straw was improved by adding buckwheat husk at a C/N ratio of 10. Borowski and Kucner [6] showed that increasing the manure by content by 20% can improve CH_4 production from the anaerobic co-digestion of SBP and sludge at an organic loading rate of 4.25 kg VS/m^3.d. Babaee et al. [4] studied the co-digestion of manure and WS,they reported that CH4 production was increased by 43% at a temperature of 35 °C. Aboudi et al. [1] studied the semi-continuous digestion of sugar beet by-product with manure, the result showed that the optimal CH_4 production was conducted at an organic load of 11.2 kg VS/m^3.d.

As a first step in this study, the production of CH_4 from anaerobic digestion of CM in a batch reactor was improved by adding WS and Ln at different mixing ratios. In terms of sustainability, it is important to use the residues of different crops to avoid suspending the biogas production in the reactor when a certain crop is out of season; this will be of enormous benefit to the industry. In a second step, since the effects of transient co-substrate changes using different waste materials have been poorly documented in previous works, this study also investigated the effects of a transient change in the co-substrate in multi-substrates on gas production and reactor performance, using a semi-continuous stirred tank reactor.

2 Methodology

2.1 Preparation of substrates

Cow manure (CM) was acquired from a small farm in Coueron (GAEC des Marais, France), homogenized and stored at -3 °C for later use. SBP, WS and Ln were obtained from a farm in Nantes (France) and ground with a Retsch SM 300 cutting mill (Germany) to reduce particle size to below 1.0 mm for optimum CH_4 production, as recommended by Yong et al. [27].

2.2 Inoculum

For this work, the inoculum was used from the IMT Atlantique reactor (GEPEA laboratory, Nantes, France). The sludge was obtained from the Saint-Nazaire (France) wastewater treatment plant, comprising 60% digested sludge and 40% activated sludge. The original temperature of the inoculum in the reactor was 37 °C.

2.3 Analytical techniques

A Flash EA 1112 (Thermo Finnigan, IMT Atlantique, France) was used to analyze the elements (C, N, H, O) in this study. The volatile solids, total solids, and pH were analyzed using APHA Standard Methods [3]. The biogas production rate was analyzed by the water displacement method, using an Agilent Innovations G2801A (China). The cumulative biogas production was assessed to STP values (10^5 Pa and 273.15 K). The characteristics of the substrate and inoculum are shown in Table 1.

Table 1 Characterization of feedstock and inoculum

Characteristics	CM	SBP	Ln	WS	Inoculum
VS (TS %)	65.91 ± 0.13	96.22 ± 0.13	98.20 ± 0.10	94.23 ± 0.12	81.97 ± 0.08
TS (dry wt. %)	6.79 ± 0.12	85.00 ± 0.36	88.42 ± 0.15	88.33 ± 0.18	4.123 ± 0.36
TC (dry wt. %)	38.81 ± 0.32	41.17 ± 0.30	48.64 ± 0.44	46.50 ± 0.58	ND
TN (dry wt. %)	2.80 ± 0.16	2.4 ± 0.12	0.59 ± 0.25	0.33 ± 0.04	ND
TO (dry wt. %)	30.20 ± 0.15	46.11 ± 0.02	28.30 ± 0.19	42.35 ± 0.42	ND
TH (dry wt. %)	6.10 ± 0.12	6.54 ± 0,34	5.98 ± 0.09	6.14 ± 0.17	ND
pH	8.50 ± 0.15	ND	ND	ND	7.08 ± 0.09
C/N ratio	13.86	17.15	82.44	140.91	ND

Notes: *VS* volatile solids, *TS* total solids, *TC* total carbon, *TN* total nitrogen, *TO* total oxygen, *TH* total hydrogen, *C/N* nitrogen to carbon ratio. The data represent the mean ± SD, $n = 3$

2.4 Experiment design and set-up

3 Biochemical methane potential (BMP) test

The biochemical methane potential (BMP) test was carried out first, in triplicate, using 500 mL bottles and under mesophilic conditions, based on the method described by Elsayed et al. [8]. The anaerobic co-digestion of CM, Ln and WS was carried out using various mixing ratios of 100/00/00, 70/15/15, 50/25/25, 34/33/33, 25/50/25, 25/25/50, 00/100/00, and 00/00/100 respectively, to obtain the best mixing ratio for high gas production (Table 2).

4 Semi-continuous reactor

The semi-continuous co-digestion of CM, Ln and WS or SBP was carried out using a stainless steel semi-continuous stirred tank reactor (SSTR-MP30) (Fig. 1). The total volume of the SSTR was 75 L and the maximum available working volume 50 L. The temperature of the SSTR was controlled using a coolant-circulating jacket to ensure mesophilic conditions for the bacterial activity (37 ± 1 °C). The reactor had a light-up window for viewing the processed substrate inside the tank. The substrate was fed into the reactor by two peristaltic pumps and mixing in the reactor was controlled using a marine propeller agitator.

To monitor the effects of the transient co-substrate change on anaerobic co-digestion (using the optimal mixing ratio obtained in the BMP test), three runs were carried out. For run 0, the SSTR reactor was loaded with inoculum alone for 10 days, to activate micro-organisms under mesophilic conditions [18]. In run 1, semi-continuous co-digestion of CM, Ln and WS was carried out with a 35 L working volume and an organic loading rate (OLR) of 1 kgVS/m^3. d (37° C ± 1). In run 2, semi-continuous co-digestion of CM, Ln and SBP was carried out, replacing the WS co-substrate with SBP, to examine the effects that changing the co-substrate had on the biogas production rate and biodegradability of the substrates used in multi-substrates (Table 3). The hydraulic retention time of

Table 2 Anaerobic co-digestion in batch reactor of CM, Ln and WS at different mixing ratios

Batch reactor number	CM (gVS/400 mL)	Ln (gVS/400 mL)	WS gVS/400 mL)	Mixing ratio (CM/Ln/WS)
T1	5.25	1.13	1.13	70/15/15
T2	3.75	1.88	1.88	50 /25/25
T3	2.55	2.48	2.48	34/33/33
T4	1.88	3.75	1.88	25/50/25
T5	1.88	1.88	3.75	25/25/50
C1	7.5	0.00	0.00	100/00/00
C2	0.00	7.5	0.00	00/100/00
C3	0.00	0.00	7.5	00/00/100

CM cow manure, *Ln* linen, *WS* wheat straw

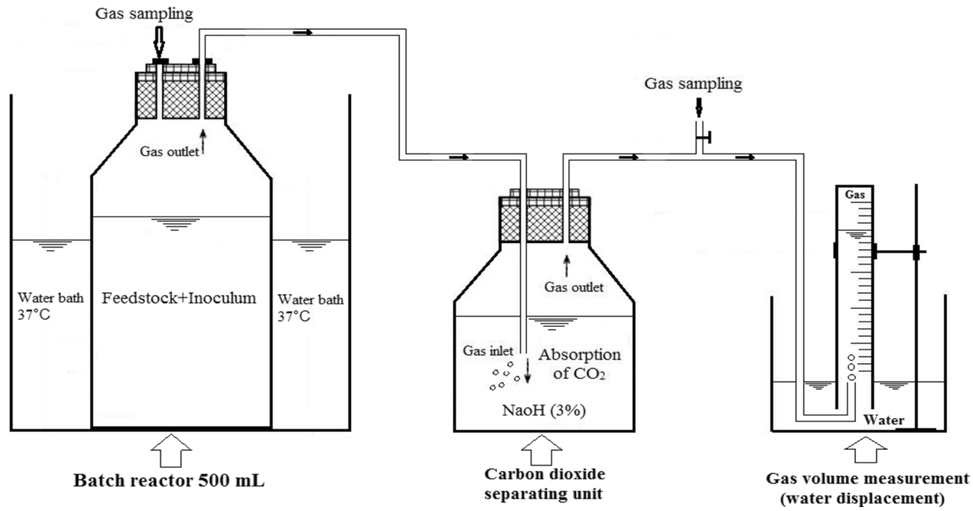

Fig. 1 Batch reactor test set-up [8]

Table 3 Characteristics of transient co-substrate change in semi-continuous co-digestion of CM, Ln and WS or SBP

Run	CM (kgVS)	Ln (kgVS)	WS (kgVS)	SBP (kgVS)	OLR (kgVS/ m^3. d)	HRT (days)	Mixing ratio
Run 0	0.00	0.00	0.00	0.00	0.0	10	0.00
Run 1	122.5	61.25	61.25	0.00	1.0	15	50:25:25
Run 2	122.5	61.25	0.00	61.25	1.0	15	50:25:25

Notes: *CM* cow manure, *Ln* linen, *WS* wheat straw, *SBP* sugar beet pulp, *OLR* organic loading rate, *HRT* hydraulic retention time

15 days was kept constant for the two steps, feeding the reactor with 2.33 L of feedstock (substrates + water) and removing 2.33 L from the reactor each day.

In expansion, approximately 100 mL of the digestate was established every 3 days before feeding the reactor, to assess the biodegradability of the substrates. The CH_4 content was analyzed twice a week for the amount of biogas produced (Fig. 2).

4.1 Kinetic analysis of cumulative biogas production

The modified Gompertz equation (Eq. 1) proposed by [22] is used to describe the kinetics of methane production. This model has been used by several authors where the biogas production has a lag phase, enabling prediction of the adaptation phase prior to methane production, when the substrate presents a high concentration of the less-biodegradable compounds [10, 11, 19].

$$H(t) = P.exp\left[-exp\left[\frac{R_m.e}{P}(\lambda - t) + 1\right]\right] \tag{1}$$

where H (t) is the accumulative methane production (mL/g_{vsadd}), P the methane production potential (mL/g VS_{add}), Rm the maximum methane production rate (mL/g VS_{add}/day), λ the lag-phase time (days) and e = 2.718281828.

4.2 Statistical analysis

For this study, statistical analysis was carried out using ANOVA analysis, the tested conditions were compared using STATGRAPHICS Centurion XV software (Virginia, USA), and the differences in biogas production with various fractions of CM, Ln and WS were analyzed at a confidence interval of 95%.

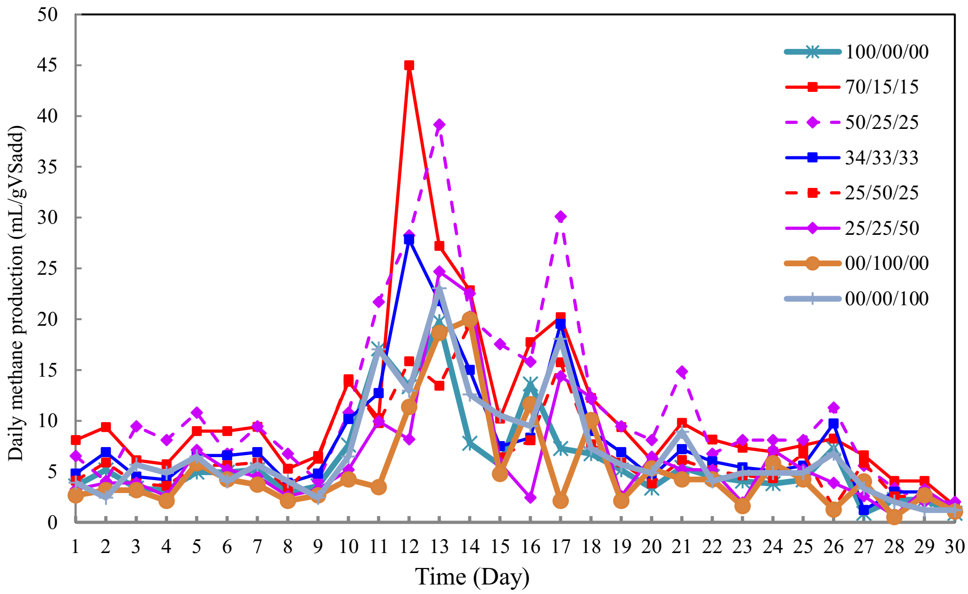

Fig. 2 MP30 Methanization reactor

5 Results and discussion

5.1 Anaerobic co-digestion of CM, Ln and WS in a batch reactor

6 CH_4 production

Daily CH_4 yields from the co-digestion of CM, Ln and WS at different mixing ratios using the batch reactor are shown in Fig. 3. The peak values at mixing ratios of 100/00/00, 70/15/15, 50/25/25, 34/33/33, 25/50/25, 25/25/50, 00/100/00 and 00/00/100 were 19.8, 45, 39.2, 27.8, 19.5, 24.7, 20 and 23 mL/g VS_{add}, respectively, obtained mainly between the day 11 and day 15 of AD. The highest peak was recorded at the mixing ratio of 70/15/15 on day 12 from the start of the BMP test, while the lowest value was recorded at the mixing ratio of 25/50/25 on day 14. This may be because the mixing ratio of 70/15/15 contained a high percentage of CM and lower percentages of Ln and WS; these agricultural wastes contain cellulose and other non-digestible matter, which it is not easily degraded by micro-organisms [10, 11, 23].

The cumulative methane yields (CMYs) from co-digestion of CM, Ln and WS at normal temperature and pressure (N) conditions are shown in Fig. 4. The CMYs from co-digestion at mixing ratios 100/00/00, 70/15/15, 50/25/25,

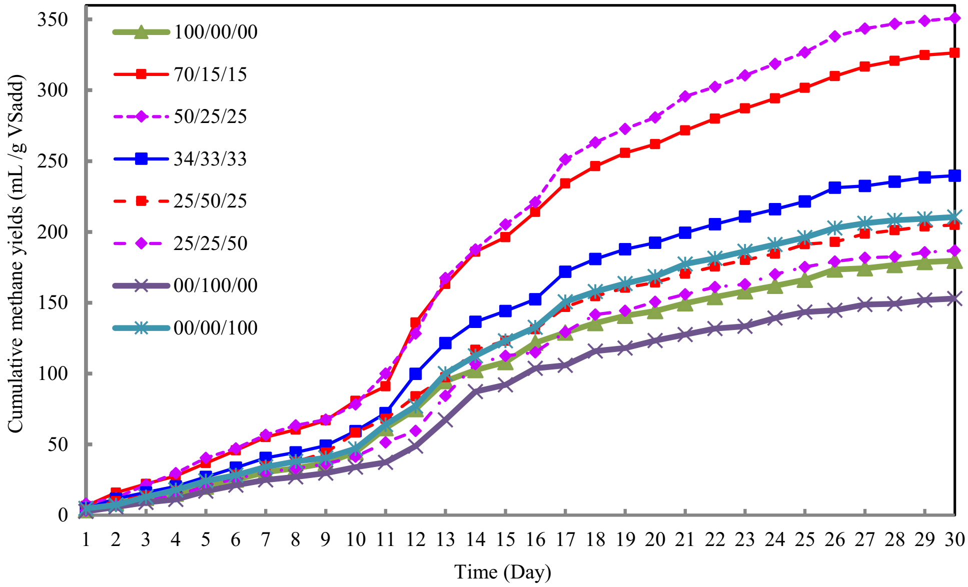

Fig. 3 Daily CH4 production from co-digestion of CM, Ln, and WS

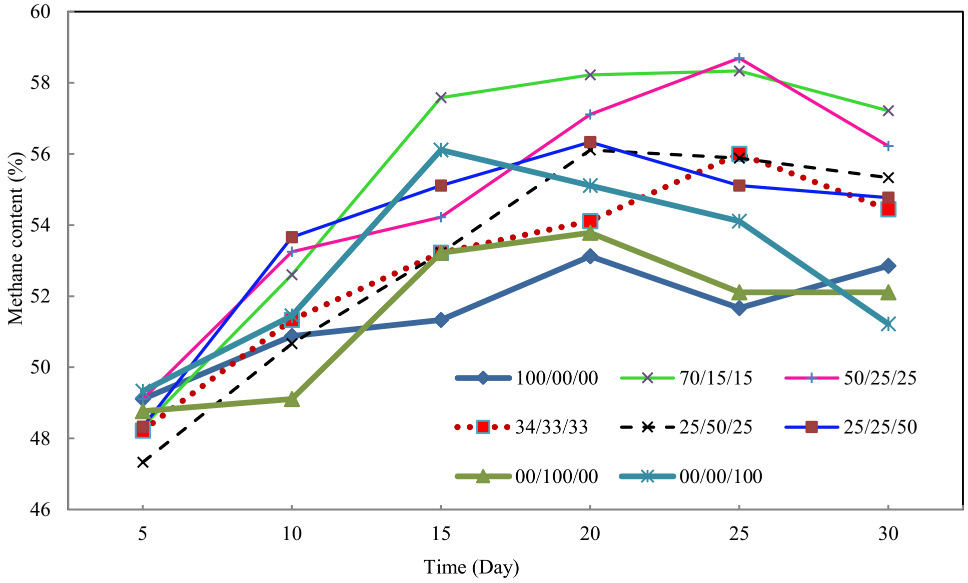

Fig. 4 CMYs from co-digestion of CM, Ln and WS

34/33/33, 25/50/25, 25/25/50, 00/100/00 and 00/00/100 were 180, 326, 351, 240, 205, 187, 153 and 211 mL/g VS_{add}, respectively. The CMYs observed with the various mixing ratios were higher than those for individual digestion of the feedstock used. An analysis of variance (ANOVA) test on the cumulative methane yields (CMYs) for co-digestion tests showed a P-value for the F-test of less than 0.05, with a statistically significant difference between the mean cumulative methane from one CM/Ln/WS mixing ratio to another at a confidence level of 95%. A comparison of mixing ratios showed that CMYs were higher with an increase in CM percentage. The mixing ratio of 50/25/25 is statistically the optimum for high methane production. This mixing ratio contains a low percentage of hemi-cellulose and lignin. Hemi-cellulose and lignin are not easily biodegradable [25] due to the stability of cellulose microfibers and the polysaccharidic coating [2]. However, the lowest CMYs were observed at the mixing ratios 25/25/50 and 25/50/25.

7 CH_4 content and VS removal rate

The methane (CH_4) content from co-digestions of CM, Ln, and WS is shown in Fig. 5. The highest average CH_4 percentages were observed at the mixing ratios 70/15/15 and 50/25/25, while the lowest value was at the ratio 25/50/25. However, the CH_4 percentages for the various mixes were higher than those obtained from individual digestion of the feedstock used. A comparison of the various mixing ratios shows that the CH_4 content was higher when the CM percentage in the ratio was increased.

The VS removal rates and pH values for co-digestion of CM, Ln and WS are shown in Fig. 6. The VS removal rates increased more gradually at the mixing ratios 50/25/25 and 70/15/15 than at the other ratios. The lowest VS removal rate was recorded at the mixing ratio 25/50/25. Finally, the pH values ranged between 7.11 and 7.52, which is considered an acceptable range for micro-organism growth [21].

8 Kinetic analysis of cumulative biogas production at different CM/Ln/WS ratios

Figure 7 represents the estimated and observed CMYs from anaerobic co-digestion of CM, Ln and WS at different mixing ratios. The curves were estimated using Eq. 1, which predicts two-phase anaerobic digestion: an initial phase of biogas production from the easily-biodegradable material, and a second phase of degradation of the material after it has been subjected to a biological hydrolysis step, and with a time lag λ between the two phases [10, 11]. As a first observation, this model provides a good description of the AD of the various mixes,the presence of an agricultural substrate in the mix explains the inflection point corresponding to the lag phase prior to biogas production.

The parameters of the modified Gompertz equation are set out in Table 4. The low RMSE values show that the CMYs observed are closely aligned with the theoretical values. Table 4 also shows the lag times of between 4 and 5 days observed for the various mixes tested, demonstrating that this parameter depends more on the nature of the substrates than on their percentage in the mix. In cases using other types of substrates, such as activated sludge, longer lag times of around 15 days have been observed [10, 11], confirming this result. It is also observed that maximum biogas productivity is obtained for the 50/25/25 mix, with an estimated CMY value of 378.6 mL/g VS_{add} and a maximum methane production rate (Rm) of 20.02 ml $/gVS_{add}$/day. The model also provides for higher biogas production when the CM concentration is higher; the low kinetic parameters were obtained under conditions where the CM concentration was zero. Given the high nitrogen concentration in the CM (Table 1), this result shows the effects of this substrate in the C/N mixing ratio, producing the most favourable conditions for optimal microbiological activity.

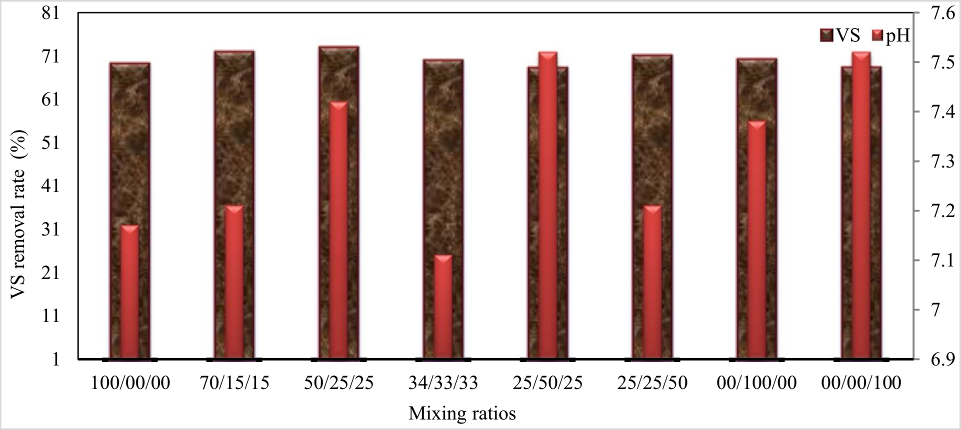

Fig. 5 Average CH4 content from co-digestions of CM, Ln, and WS

Fig. 6 VS removal rates and pH values from co-digestions of CM, Ln and WS

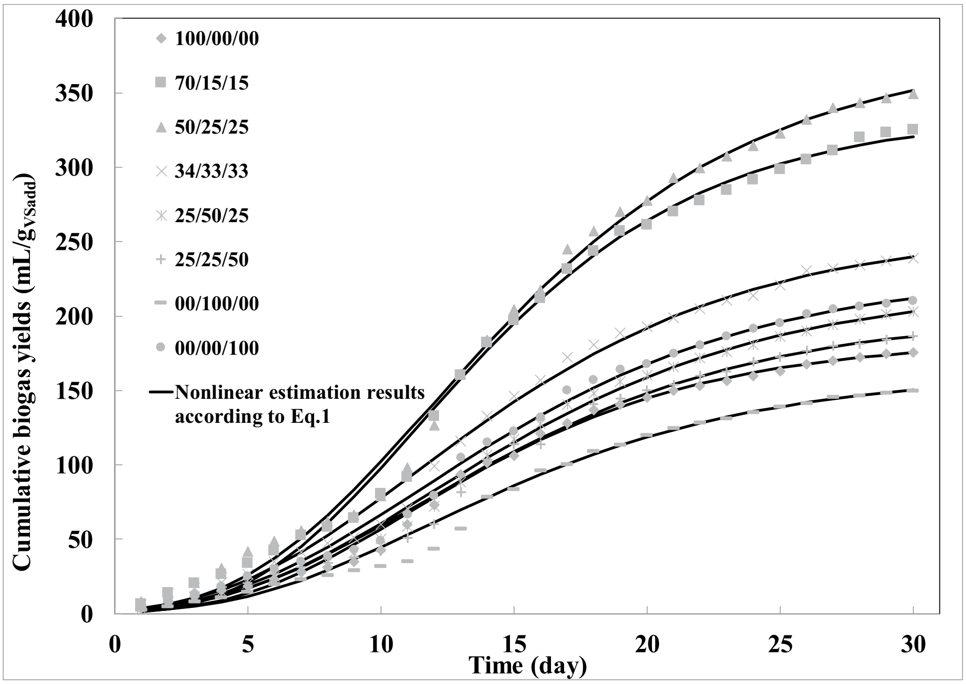

Fig. 7 Estimated and observed CMYs from anaerobic co-digestion of CM, Ln and WS at different mixing ratios (CM/Ln/WS)

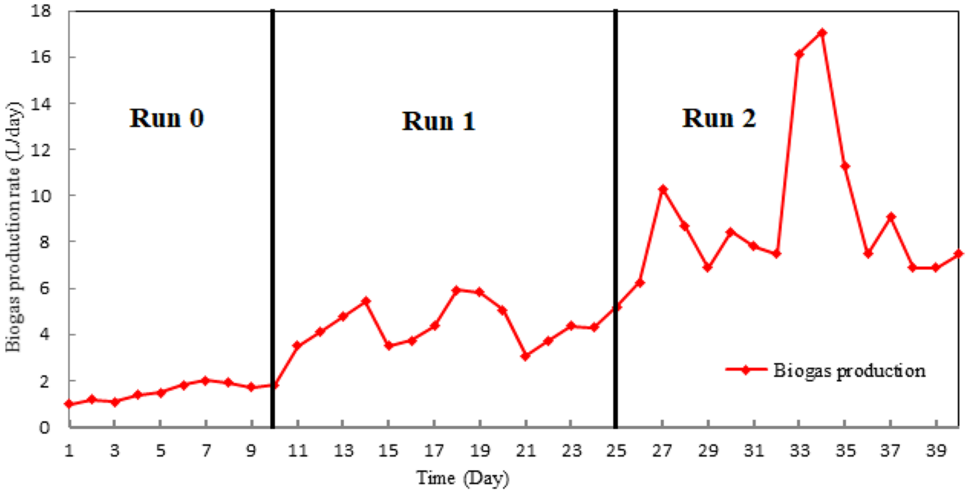

Table 4 Kinetic parameters of BMP tests calculated from non-linear regression of Eq. 1

Mixing ratio (CM/Ln/WS)	P (ml/gVS$_{add}$)	Rm (ml/gVS$_{add}$/day)	Lamda (Day)	RMSE
100/00/00	184.35	10.72	4.72	2.452
70/15/15	336.13	20.20	5.16	4.834
50/25/25	378.62	20.02	4.92	4.715
34/33/33	257.71	13.33	4.19	3.206
25/50/25	220.40	11.14	4.59	2.352
25/25/50	201.22	10.27	4.28	3.005
00/100/00	161.83	8.48	4.78	2.383
00/00/100	228.95	11.65	4.33	2.681

8.1 Semi continuous co-digestion of CM, SBP, Ln, and WS

9 Effects of transient change of co-substrate for multi-digestion

The effects of a transient change in co-substrate for multi-digestion using different waste materials are shown in Fig. 8. In this part, three runs were conducted. In the initial step (run 0), the lowest daily biogas yield was observed when the SSTR reactor was fed with inoculum only. In run 1, the semi-continuous co-digestion of CM, Ln and WS was

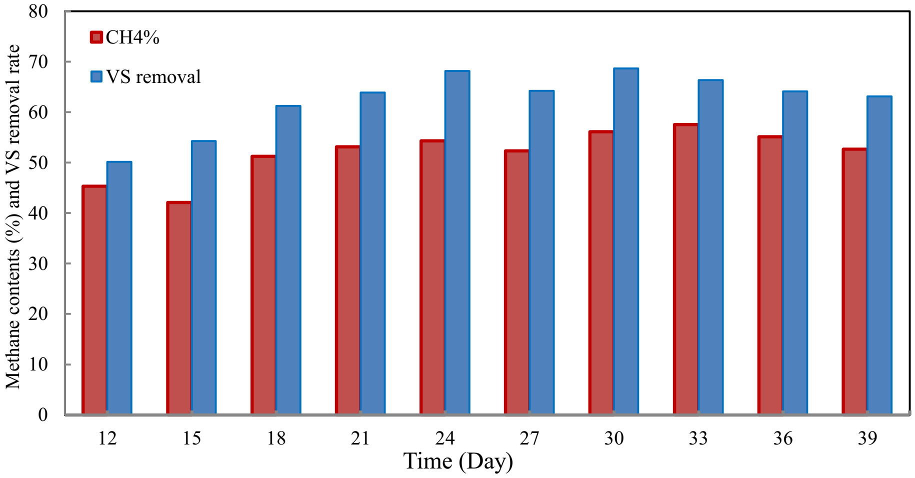

Fig. 8 Daily biogas yields for semi-continuous co-digestion of CM, Ln and SBP (or WS)

carried out using the organic loading rate (OLR) of 1 kgVS/m^3. d. In this stage, the daily biogas yields increased more gradually than in the initial step, as a result of feeding the reactor with CM, Ln and WS. The highest daily biogas yields from co-digestion of CM, Ln and WS were 5.93 and 5.81 L/d, observed on days 18 and 14 respectively. In run 2, the co-substrate WS was replaced with SBP to examine the effects of changing the co-substrate (in multi-substrates) on biogas yields and biodegradability. In this stage, the daily biogas yields increased more gradually than in run 1 (where WS was used as co-substrate). The highest daily biogas yields from co-digestion of CM, Ln and SBP were 17.06 and 16.13 L/d, recorded on days 34 and 33 respectively, a yield 2.88 and 2.78 times higher than the highest values observed in run 1 (biogas yields two times higher than the values recorded in run 1). In general, a transient change of co-substrate using different waste materials and multi-substrates improves biogas yields and increases the sustainability of gas production throughout the year, since harvesting seasons demand that different types of crop are used. For this study, we started the semi-continuous co-digestion of CM and Ln with the abundant crop WS; for the second step, we replaced WS with SBP, also considered an abundant crop, to study the effects of a transient change of co-substrate on biogas production. However, WS was the only substrate replaced with SBP, in order to maintain the stability of the reactor.

Finally, it is important to use the residues of different crops in season to avoid suspending biogas production in the reactor. This will be of enormous benefit to the industry. This result coincides with previous studies: Fonoll et al. [16] studied the effects of substituting different types of fruit with sludge for gas production, compared with mono-digestion of the fruits. The results showed that changing one kind of fruit with the same type did not cause system failure. In this study, however, we examined the effects of a transient change of co-substrate (for multi-substrates) on biogas production and system stability.

The VS removal rate and methane (CH_4) content for the transient change of co-substrate are shown in Fig. 8. CH_4 content increased slightly with a change in co-substrate from WS (in run 1) to SBP (in run 2). The highest CH_4 content of 54.33% (day 24) and 57.54% (day 33) were observed in runs 1 and 2 respectively. In addition, the VS removal rate increased gradually when the co-substrate was changed from WS (Run 1) to SBP (Run 2). The maximum VS removal rates of 68.14% and 68.64% were achieved in runs 1 and 2 respectively. The results show that a transient change of co-substrate from WS to SBP has a positive effect on VS removal rate and CH_4 content, improving them both.

10 Conclusion

This work reports on the sustainability of improving CH_4 production from the co-digestion of CM, SBP, Ln and WS based on their mixing ratios and a transient change of co-substrate. A BMP test was first carried out to ascertain the mixing ratio for highest gas production from the co-digestion of CM, WS and Ln. The results show first of all the best CH_4 production at a mixing ratio of 50/25/25, with a value of 351 mL/g VS_{add}. However, VS removal rates and CH_4 content were shown to gradually increase at mixing ratios of 50/25/25 and 70/15/15 compared to the other ratios. These results are confirmed by the kinetic study. In the subsequent experiments, the semi-continuous co-digestion of CM, SBP, Ln, and WS was carried out to study the effects of transient change in operating parameters on gas production and reactor performance. The advantages of this study are the sustainability of CH_4 production in the off-season, which will be a great advantage for the industry. The results show that a transient change of co-substrate in multi-substrates could double the daily CH_4 production when the co-substrate is changed from WS to SBP, and that CH_4 production is therefore sustainable.

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