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ORIGINAL PAPER

Evaluation of Semi-dry Mesophilic Anaerobic Co-digestion of Corn Stover and Vegetable Waste by a Single-Phase Process

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Abstract The performance of semi-dry anaerobic co-digestion of corn stover and vegetable waste was investigated using batch reactors and leaching bed reactors (LBRs). Six mixing ratios of corn stover and vegetable waste were employed to investigate optimal methane production using batch reactors, and process stability was assessed using the two LBRs. Kinetic assessment, methane production, volatile solid (VS) reduction, and digestate characteristics were used to evaluate the performance of anaerobic co-digestion. After 50 days of anaerobic co-digestion in the batch reactor, methane yields reached 314.5–323.4 mL/g VS, and increase in methane production rate indicated that synergistic effects occurred during anaerobic co-digestion. The corn stover and vegetable waste with a total solids (TS) ratio of 14:1 yielded the highest methane production efficiency and rate, as well as VS removal efficiency. However, serious acidification at the initial stage of digestion in the LBR experiments suggests that the scaling-up process from batch reactor to LBR was unstable, which may be attributed to high TS concentration and substrate characteristics.

Keywords Anaerobic co-digestion · Methane production · Process stability · Mixing ratio · Kinetic assessment · Acidification

Introduction

Interest in anaerobic digestion of lignocelluloses for bioenergy production has surged in recent years among researchers worldwide. Corn yield throughout China exceeded 205.6 million tons in 2012 according to the China Statistical Yearbook 2013 [1]. The vast amount of corn stover generated from corn cultivation in China is either burned or abandoned in open fields, causing serious environmental pollution. To address this issue, anaerobic digestion of corn stover for bioenergy production has been proposed [2]. Despite obvious benefits of the procedure, some characteristics of corn stover, such as its lignocelluloses structure, result in poor methane production efficiency and low hydrolysis rate [2, 3].

Another potential resource that can be used for bioenergy production is vegetable waste. Annually, up to 1.3×10^7 ton of vegetable waste is generated during vegetable processing and transport in cities in China [4]. Vegetable wastes are characterized as easily biodegradable organic matter with high moisture contents and low C/N ratios [5, 6]. Anaerobic mono-digestion of vegetable waste is limited by rapid acidification [7], which causes failure of the anaerobic digestion process. As the substrate characteristics of corn straw and vegetable waste complement each other, these materials may be anaerobically co-digested to balance the C/N ratio, eliminate VFA accumulation, and increase the methane production efficiency of corn stover. Therefore, the performance of anaerobic digestion can potentially be boosted by anaerobic co-digestion of corn stover and vegetable waste.

Anaerobic co-digestion of lignocelluloses with other easily biodegradable waste has been considered to be a promising technology for improving the digestibility of lignocelluloses [8]. Zhong et al. [9] found that anaerobic co-digestion of blue algae and corn stover increased methane productivity

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by 46% and improved organic loading rates for the anaerobic process, because anaerobic co-digestion increases hydrolysis and methanogenic rates by eliminating volatile fatty acid (VFA) buildup and reducing free ammonia (FA) content. Similarly, efficiency and stability of the anaerobic process can be improved by co-digestion of corn stover and chicken manure [10]. Despite these promising results, the efficiency of anaerobic digestion process is largely dependent on the characteristics and type of co-digested biomasses. To the best of our knowledge, studies on anaerobic co-digestion using corn stover and vegetable waste have not been reported to date.

In general, semi-dry anaerobic digestion is defined based on total solid (TS) concentration of 10–15% [10, 11]. Compared with wet and dry anaerobic digestion, semi-dry anaerobic digestion not only has high mass transfer between organic matter and anaerobic microorganisms but also reduces water consumption and wastewater generation [12]. Therefore, semi-dry anaerobic co-digestion of corn stover and vegetable waste was carried out in the present study.

The main goal of this study was to investigate the feasibility of semi-dry anaerobic co-digestion of corn stover and vegetable waste. Six mixing ratios of corn stover and vegetable waste were anaerobically co-digested to achieve optimal methane production in batch reactors. The process stability of anaerobic digestion was assessed in two leaching bed reactors (LBRs). Comparisons of methane production and VS reduction, as well as digestate characteristics, were conducted. The modified Gompertz model was used to simulate methane production.

Materials and Methods

Corn Stover, Vegetable Waste, and Anaerobic Seed Cultures

Corn stover was collected from Nongcui Garden of Anhui Agricultural University, China, in September 2016 and chopped into 3–4 cm pieces with shears after air-drying. Pak choi (*Brassica chinensis* L. cv. "Shanghaiqing") was purchased from a supermarket as vegetable waste and blended to homogeneity in a food blender before anaerobic digestion. The inoculums were well-digested rice straw obtained from our laboratory [13]. Anaerobic culture was concentrated by settling and used as inoculums. The chemical characteristics of the feedstock and anaerobic culture are shown in Table 1.

Table 1 Chemical characteristic of the feedstock and anaerobic culture

	Corn stover	Vegetable wastes	Anaerobic inoculums
TS (%)	89.37 ± 1.18	2.88 ± 0.08	4.10 ± 0.03
VS (%TS)	92.69 ± 1.27	72.61 ± 2.15	41.80 ± 0.03
C (%)	43.9	40.69	/
N (%)	1.6	4.45	/
H (%)	5.92	5.84	/
S (%)	0.22	0.54	/
C/N	27.44	9.14	/
Cellulose (%)	28.15	25.29	/
Hemicellulose (%)	15.34	7.30	/
Lignin (%)	10.38	0.76	/
pH	/	/	7.53

Anaerobic Co-digestion Experiment

Anaerobic co-digestion was conducted in 12 batch reactors (1 L jars) and 2 identical LBRs. The inner diameter of the LBR was 14 cm, its height was 50 cm, and its working volume was 4.0 L Detailed descriptions of the batch reactor and LBR were provided elsewhere [12, 13].

A detailed experimental design of anaerobic digestion is given in Table 2. The initial TS concentrations of reactor for anaerobic co-digestion were approximately 10%, whereas those for mono-digestion of vegetable waste and corn stover were 3.6 and 12.1%, respectively. These may be due to the high water content of vegetable waste and high TS content of corn stover. The C/N ratio of digested substrate should be in the range of 20:1–30:1 for anaerobic digestion [3]. To keep the C/N ratio of 20–30 and reactor TS concentration of about 10%, six mixing ratios of corn stover and vegetable waste (i.e., 0:1 (R1), 1:0 (R2), 5.5:1 (R3), 8:1 (R4), 11:1 (R5), and 14:1 (R6) (TS, w/w)) were selected. The C/N ratio of feedstock was 27.4 (R2), 21.2 (R3), 22.7 (R4), 23.7 (R5), and 24.4 (R6), respectively, and mono-digestion of vegetable waste (R1) had a C/N value of 9.1.

For 1 L batch experiments, appropriate amounts of corn stover and vegetable waste were mixed with 350 mL of anaerobic seed cultures in a 1 L jar, and distilled water was added to achieve the desired TS concentration. Then the feedstock was uniformly mixed. The reactor was finally sealed with butyl rubber stoppers after flushing with nitrogen gas for 2 min. Each bottle was manually mixed twice a day to avoid stratification during the 50 days anaerobic digestion period. Each experiment was conducted in duplicate. Methane yield under standard conditions is reported.

The result of 1 L batch experiment showed that the experimental group with a TS ratio of 14:1 had optimal performance. Therefore, in the LBR experiments, two TS ratios of corn stover and vegetable waste [1:0 (LBR1) and 14:1 (LBR2)] were selected to determine changes in process stability. For anaerobic digestion of LBR, corn stover and/or vegetable waste, and 1000 mL of anaerobic cultures were added to the bucket, and the appropriate volume of

Table 2 Experimental design on semi-dry anaerobic co-digestion of corn stover and vegetable wastes

Experimental groups	R[a]	Anaerobic inocula (mL)	Distilled water (mL)	Total TS weight[b] (g)	C/N[c]	TS concentration[d] (%)
R1	0:1	350	0	7.2	9.1	3.6
R2	1:0	350	177	57.2	27.4	12.1
R3	5.5:1	350	0	44.4	21.2	9.3
R4	8:1	350	45	48.2	22.7	10
R5	11:1	350	70	49.7	23.7	10.4
R6	14:1	350	88	50.8	24.4	10.7
LBR1	1:0	1000	531	125.1	27.4	9.9
LBR2	14:1	1000	264	124.8	24.3	9.8

[a] TS ratio of corn stover and vegetable wastes

[b] Calculated according to the wet weight and TS content of corn stover and vegetable wastes

[c] Calculated according to elemental analysis, wet weight and TS content of corn stover and vegetable wastes

[d] TS concentration of reactor

distilled water was added to achieve a TS concentration of 10%. Anaerobic digestion in the LBR was conducted for 45 days. During anaerobic digestion, 450 mL of the leachate was recycled to the top of the reactor by spraying the bed twice daily [13]. Biogas production was recorded daily by a wet tip gas meter and reported in units of liters per day under standard conditions. Biogas production and composition, as well as pH, COD, VFA, NH_4^+–N, and TA from the LBR leachate, were analyzed during anaerobic digestion.

Kinetic Assessment and Calculations

The modified Gompertz model has been widely used to describe hydrogen and methane production [14] and is expressed as indicated in Eq. (1) below.

$$M = P \times \exp\left(-\exp\left[\frac{R_m \times e}{P}(\lambda - t) + 1\right]\right) \quad (1)$$

where M is the cumulated methane yield at time t, P is the potential methane production, R_m is the maximum rate of methane production, $e = 2.718281828$, λ is the lag time, and t is the measured time. P, R_m, and λ were estimated by non-linear fitting using Origin 8.0.

The predicted methane yield was calculated for different mixing ratios of corn stover and vegetable waste using Eq. (2).

$$PMY = \frac{X_1 TS_1 + X_2 TS_2}{TS_1 + TS_2} \quad (2)$$

where PMY is the predicted methane yield of anaerobic co-digestion, X_1 and X_2 are respectively the experimental methane yields of corn stover and vegetable waste by anaerobic mono-digestion from 1 L batch experiment, and TS_1 and TS_2 are the TS weights of corn stover and vegetable waste added into the reactor, respectively.

Analytical Methods

Methane content was determined by gas chromatography (Ruipu SP-6890, China). Standard methods were used to measure the concentrations of TS, volatile solids (VS), COD, and NH_4^+–N [15]. Free ammonia (FA) concentration was calculated using a previously reported method [16]. VFA and TA were determined by previous methods [12]. Elemental C, H, N, and S contents were determined using an elemental analyzer (Vario EL Cube, Germany). Neutral detergent fiber, acid detergent fiber, and acid detergent lignin were analyzed in triplicate according to a previously reported method [17].

Statistical Analysis

One-way ANOVA with Fisher's least significant difference (LSD) test was performed to evaluate significant differences at $p < 0.05$ using SPSS 14.0 for Windows.

Results and Discussion

Methane Production and Kinetic Assessment from Batch Experiment

The methane production efficiencies of semi-dry anaerobic digestion of corn stover and vegetable waste achieved in the 1 L batch reactors are summarized in Table 3. After 50 days of semi-dry anaerobic mono-digestion, methane yields reached 638.3 mL/g VS for vegetable waste and 289.5 mL/g VS for corn stover. These methane yields of vegetable waste

Table 3 Comparison in the methane production efficiency of anaerobic digestion of corn stover and/or vegetables wastes

Experimental groups	R1	R2	R3	R4	R5	R6
Specific biogas yield (mL/g TS)	559.2 ± 10.5^{a}	423.8 ± 38.2^{b}	449.7 ± 14.6^{b}	447.8 ± 12.2^{b}	457.5 ± 6.4^{b}	447.3 ± 19.4^{b}
Specific biogas yield (mL/g VS)	970.1 ± 18.3^{a}	457.2 ± 41.2^{b}	514.9 ± 16.7^{b}	504.1 ± 14.2^{b}	509.6 ± 7.1^{b}	494.2 ± 21.5^{b}
Specific methane yield (mL/g TS)	367.9 ± 12.6^{a}	268.4 ± 31.8^{b}	282.4 ± 15.2^{c}	279.4 ± 8.8^{c}	286.9 ± 3.6^{c}	286.9 ± 9.0^{c}
Specific methane yield (mL/g VS)	638.3 ± 21.8^{a}	289.5 ± 34.3^{c}	323.4 ± 17.3^{b}	314.5 ± 10.3^{b}	319.6 ± 3.9^{b}	317.7 ± 9.9^{b}
PMY[a] (mL/g TS)			283.9	279.4	276.7	275.2
EMY/PMY[b]			0.99	1.0	1.04	1.04
Mean methane production rate[c] (L/(L days))	0.14 ± 0.01^{c}	0.54 ± 0.06^{ab}	0.44 ± 0.02^{b}	0.46 ± 0.01^{b}	0.55 ± 0.02^{ab}	0.62 ± 0.03^{a}
Mean methane content (%)	65.8 ± 1.0^{a}	63.2 ± 1.8^{a}	62.8 ± 1.3^{a}	62.4 ± 0.3^{a}	62.7 ± 0.1^{a}	64.2 ± 0.8^{a}
T_{80}[d] (days)	19.9 ± 0.5^{c}	28.3 ± 1.2^{ab}	29.1 ± 0.3^{a}	29.5 ± 0.3^{a}	26.7 ± 0.6^{ab}	23.9 ± 0.8^{b}
TS removal efficiency[e] (%)		85.7^{b}	86.7^{b}	86.0^{b}	86.5^{b}	88.2^{a}
VS removal efficiency[e] (%)		86.1^{b}	86.0^{b}	86.7^{b}	86.8^{b}	88.7^{a}

Superscript a, b, c; the same row having the different letters is significantly different ($p < 0.05$) using LSD

[a] *PMY* predicted methane yield calculated according to the methane yields of corn stover and vegetables wastes by anaerobic mono-digestion

[b] The ratio of experimental methane yield (EMY) and predicted methane yield (PMY)

[c] Defined as the cumulative biogas volume at T_{80} divided by working volume and T_{80}

[d] Technical digestion time

[e] TS/ VS removal efficiency of corn stover

were higher than those obtained from 23 samples of vegetable waste ranging from 190 to 400 mL/g VS [18], and the solid-state batch anaerobic digestion of corn stover [3]. These differences observed were attributed to the diversity of substrate characteristics [19] and other factors, such as inoculum source [20] and TS concentrations of reactor. The methane yields for anaerobic co-digestion of corn stover and vegetable waste ranged from 314.5 mL/g VS to 323.4 mL/g VS. Specific methane yield of anaerobic mono-digestion of corn stover (R2 experimental group) was 289.5 mL/g VS and significantly lower than those of anaerobic co-digestion.

The PMYs for anaerobic co-digestion were calculated using Eq. (2) and ranged from 275.2 to 283.9 mL/g TS (Table 3). As shown in Table 3, the ratios of experimental methane yield (EMY) and PMY were in the range of 0.99–1.04, and increasing methane yield was not observed in the anaerobic co-digestion of corn stover and vegetable. However, an increase in mean methane production rate was found in anaerobic co-digestion compared to anaerobic mono-digestion, particularly compared to R1, suggesting synergistic effects during anaerobic co-digestion [10]. These effects may be attributed to balance of nutrients and alleviation of VFA accumulation in the reactors [8]. Moreover, the mean methane production rate increased with decreasing amount of vegetable waste. This trend may be explained by the easily degradable characteristics of vegetable waste, which produces accumulation of VFA during anaerobic digestion, leading to instability of the anaerobic digestion process [6]. The mean methane production rate of anaerobic mono-digestion of corn stover (R2) was higher than those of R3 and R4, but lower than those of R5 and R6.

The mean methane contents observed for semi-dry anaerobic co-digestion ranged from 62.4 to 65.8% (Table 3). The technical digestion time (T_{80}) refers to the amount of time during which cumulative biogas production reaches 80% of the total biogas yield [21]. The value of T_{80} in the present work ranged from 19.9 to 29.5 days. VS removal efficiencies for corn stover ranged from 86 to 88.7% after 50 days of digestion. The TS and VS removal efficiencies of R2 group were close to those of R3, R4 and R5 groups. These values were similar to those obtained for anaerobic co-digestion of livestock and vegetable processing waste (86% VS reduction) [22] but slightly higher than that for anaerobic co-digestion of fruit/vegetable waste with other organic waste (73.1–85.4% VS reduction) [5]. R6 exhibited the highest methane production efficiency and rate, as well as VS removal efficiency among the reaction systems observed; this system also yielded the lowest T_{80} among the four mixing ratios of anaerobic co-digestion studied. Thus, the optimal mixing TS ratio of corn stover and vegetable waste was 14:1.

The values of the parameters calculated by the modified Gompertz model are presented in Table 4. The coefficient of determination (R^2) was higher than 0.98 and the error rates were less than 8.4%, which indicated that the modified Gompertz model is suitable for simulating the experimental data. The maximum methane production rate (R_m) increased with decreasing amount of vegetable waste for anaerobic co-digestion, and R6 had the highest R_m compared with those of mono-digestion of corn stover and anaerobic co-digestion. The lowest lag time (λ) occurred in R6. Thus, results of kinetic assessment also indicated that

Table 4 Values of the parameters calculated by the modified Gompertz equation

Experimental groups	R_m (mL/ (days g VS))	λ (days)	R^2	P (mL/g VS)	EMY (mL/g VS)	Error (%)
R1	58.78	11.02	0.995	647.6	638.3	1.4
R2	10.73	5.78	0.994	316.0	289.5	8.4
R3	13.65	9.93	0.989	348.0	323.4	7.1
R4	13.88	10.86	0.995	333.4	314.5	5.7
R5	14.92	7.73	0.995	324.7	319.6	1.2
R6	15.25	5.68	0.996	317.7	317.7	0

R_m maximum methane production rate, λ lag time, P potential methane production, Error = (P−EMY) × 100% /P

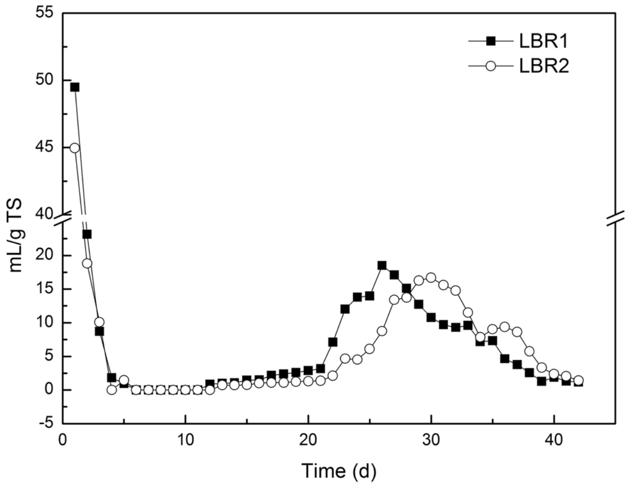

Fig. 1 Biogas production rate of semi-dry anaerobic co-digestion of corn stover and vegetable wastes by LBR

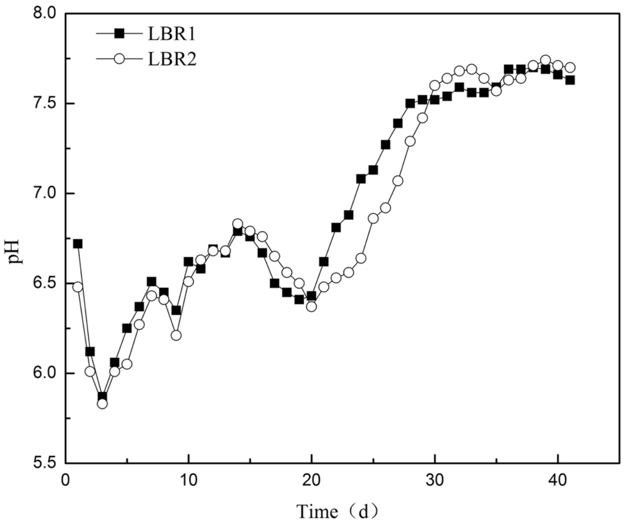

Fig. 2 Change in pH of LBR leachate from semi-dry anaerobic co-digestion of corn stover and vegetable wastes

the optimal mixing TS ratio of corn stover and vegetable waste was 14:1.

Methane Production from LBR Experiment

The profiles of specific biogas production rate in LBRs during semi-dry anaerobic digestion are presented in Fig. 1. Biogas production rates rapidly decreased in the first 5 days of digestion in both LBR1 and LBR2. After continuous alkaline (lime) supplementation for pH adjustment to 6.8 for the LBR leachate, biogas production rates gradually recovered after the 12th day, and peak values occurred on the 26th day (at 18.5 mL/(d. g TS)) for LBR1 and 30th day (at 16.7 mL/(d. g TS)) for LBR2. These results reveal severe inhibition at the initial stage of mono-digestion and co-digestion.

The specific methane yields were 185.4 mL/g VS, respectively, for LBR1 and 182.4 mL/g VS, respectively, for LBR2. The VS removal efficiency of corn stover reached 83.5% for LBR1 and 84.3% for LBR2. These values are slightly lower than those obtained from the batch reactor, suggesting that the scaling-up process from batch reactor to LBR decreased the efficiency of anaerobic digestion. The T_{80} (30.5 days) for LBR1 was lower than that for LBR2 (33.3 days). This finding suggests that the biogas production rate of anaerobic co-digestion of corn stover is lower than that of mono-digestion of the same substrate.

Process Stability of LBR Experiment

The pH profile (Fig. 2) observed during semi-dry anaerobic co-digestion reflects the relative stability of the anaerobic process. The pH profiles decreased sharply in the first 3 days of digestion from 6.72 (1st day) to 5.87 (3rd day) in LBR1 and from 6.48 (1st day) to 5.83 (3rd day) in LBR2. These profiles then gradually increased after alkaline supplementation and remained over 7.5 after the 29th day of digestion. The optimum pH for methanogens archaea ranges from 6.5 to 7.2 [23], which suggests that acidification at the initial stage of digestion inhibited methane production during the anaerobic process. No significant difference in pH was observed between the two LBRs.

VFAs are important intermediates in the anaerobic degradation of organic wastes. The COD and VFA profiles obtained during semi-dry anaerobic digestion are shown in Fig. 3. COD and VFA accumulations were observed at the initial stage of anaerobic digestion, which was the cause of rapid decrease in pH. The maximum COD values of 34.4 g/L (7th day) for LBR1 and 36.9 g/L (7th day) for LBR2 were obtained. VFAs ranged from 710 to 3960 mg/L for LBR1 and from 760 mg/L to 5910 mg/L for LBR2. There was no significant difference in VFA and COD values between the two LBRs.

The VFA and alkalinity ratio may be used to evaluate the stability of anaerobic processes [5, 24]. The TA and VFA/TA ratio profiles observed during semi-dry anaerobic co-digestion are presented in Fig. 4. Increase in TA was observed in the first 7 days of digestion, likely due to NH_4^+–N generation and alkali supplementation, and TA values ranged from 7560 to 9410 mg $CaCO_3$/L for the two LBR experiments. VFA/TA ratios remained between 0.4 and 0.75 during the first 10 days of digestion in the two LBRs, which indicates some instability upon initial digestion [24]. No significant difference in TA contents and VFA/TA ratio was found between the two LBRs.

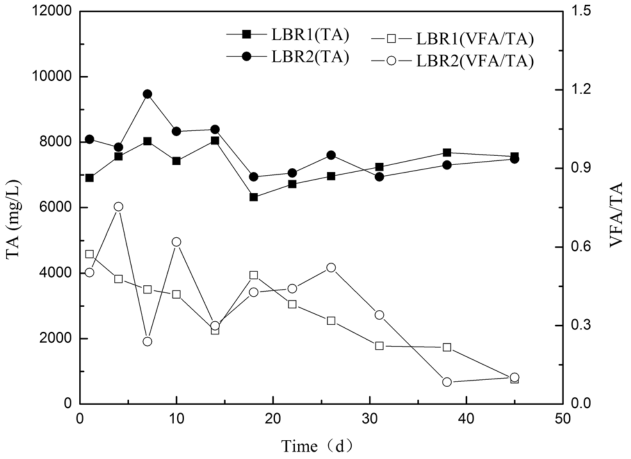

Fig. 4 Change in TA and VFA/TA ratio of LBR leachate from semi-dry anaerobic co-digestion of corn stover and vegetable wastes

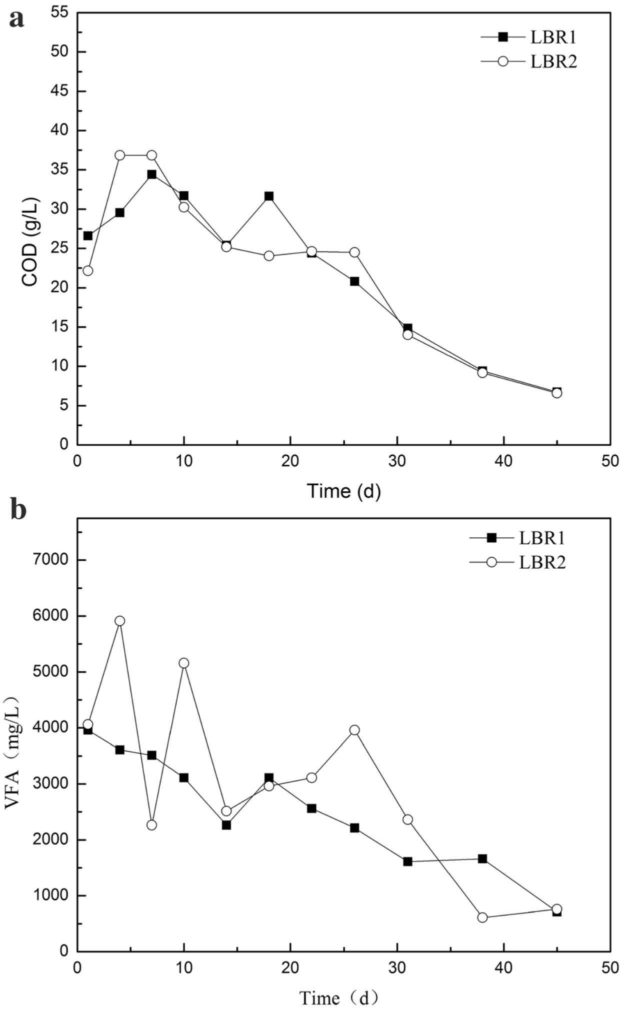

Fig. 3 Change in COD (**a**) and VFA (**b**) of LBR leachate from semi-dry anaerobic co-digestion of corn stover and vegetable wastes

High NH_4^+–N concentration can inhibit microbial activity during anaerobic digestion of vegetable waste [5]. The NH_4^+–N contents ranged from 516 to 789 mg/L for LBR1 and from 582 to 826 mg/L for LBR2 (Fig. 5). Levels of NH_4^+–N ranging from 200 to 1000 mg/L showed no antagonistic effect on the anaerobic digestion process [25]. FA is more toxic than NH_4^+–N because FA can penetrate through the cell membrane and accumulate in the cell, thereby inhibiting enzymes or causing proton imbalance [16, 25]. FA was correlated with the pH, temperature, and NH_4^+–N concentration [16], and pH values varied between 7.5 and 7.7 after 38 days of digestion. FA contents reached peak values on

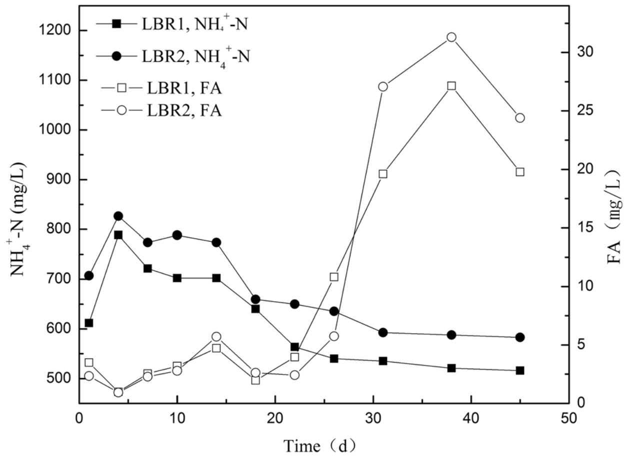

Fig. 5 Change in NH_4^+–N and FA contents of LBR leachate from semi-dry anaerobic co-digestion of corn stover and vegetable wastes

the 38th day, yielding 27.1 mg/L for LBR1 and 31.3 mg/L for LBR2. Calli et al. [26] reported that FA levels above 200 mg/L inhibit propionate-degrading acetogenic bacteria at pH 7.7 and 35 °C. Statistical analysis revealed no significant difference in NH_4^+–N and FA contents between the two LBRs.

Based on the above analysis, inhibition occurs at the initial phase of semi-dry anaerobic co-digestion at the LBR. This was due to acidification during the initial stage of digestion, as reflected by pH less than 6.0, and accumulation of COD in the two LBRs. However, no significant differences in pH, COD, VFA, NH_4^+–N, and TA levels were observed between the two LBRs. This suggested that the difference in two anaerobic processes, mono-digestion and co-digestion, was not the main causes of acidification. Therefore, the main causes of acidification were deduced from high TS concentration of semi-dry anaerobic digestion and easy biodegradation characteristics of the digested substrate.

Conclusions

After 50 days of semi-dry anaerobic co-digestion in batch reactors, methane yields reached 314.5–323.4 mL/g VS, and synergistic effects in production rate were observed during anaerobic co-digestion. The optimal TS ratio of corn stover to vegetable waste was 14:1; at this ratio, the highest methane production efficiency and production rate, and VS removal efficiency were obtained. Methane yields in LBRs were lower than those in batch reactors because of strong acidification during the initial stage of digestion, which was attributed to the high TS concentration of semi-dry digestion and easy biodegradation characteristics of digested substrates.

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