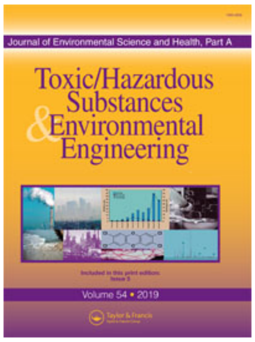

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Two-stage thermophilic anaerobic co-digestion of corn stover and cattle manure to enhance biomethane production

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ABSTRACT

Two-stage thermophilic anaerobic co-digestion of cattle manure and corn stover was conducted to increase biomethane production. The first stage pre-digestion of corn stover was studied based on the following treatment variables: corn stover to liquid fraction of digestate (CS:LFD) ratio (1:7, 1:10, 1:13, 1:14), digestion temperature (55 °C, 60 °C) and digestion time (3, 7, 14 days). The reduction in lignin, cellulose and hemicellulose (LCH) was between 3.97% and 11.98%, which increased the biodegradability of corn stover. Corn stover pre-digested with a CS:LFD ratio of 1:10 at 55 °C for a period of 3 and 7 days was subjected to anaerobic co-digestion with cattle manure. The highest biomethane yield was observed on day 21 with a value of 357.41 mL/g volatile solids (VS) for untreated corn stover, 446.84 mL/g VS for corn stover pre-digested for 3 days and 518.58 mL/g VS for corn stover pre-digested for 7 days with LFD. The VS conversion efficiency for co-digestion of cattle manure with untreated corn stover, corn stover pre-digested for 3 days and 7 days was 42.8%, 43.3% and 51.8%, respectively, on day 21, which was higher than that (34.0%) of cattle manure only.

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Introduction

Agricultural production generates large amounts of organic wastes and wastewater. Disposing or dumping these wastes back to the field can lead to reduction in crop yield, foliar diseases, and general degradation of soil quality. Meanwhile, agricultural residue can be utilized as renewable energy sources which can support sustainable products such as biofuels.[1] Therefore, cost effective technologies for disposing the organic wastes while recovering energy are necessary.[2,3]

Anaerobic digestion (AD) is an excellent waste management alternative for agricultural waste.[4,5] AD is a biological process wherein diverse groups of microorganisms breakdown complex organic wastes into simple and stable products in the absence of oxygen.[6] (AD of organic wastes has three major advantages – it provides an efficient waste treatment approach to reduce the harmful effect of wastes on the environment; energy, nutrients and water from agricultural wastes can be recycled and reused for sustainable agricultural production; and the energy contained in the biomass can be converted to biogas.[7] Biogas, a type of biofuels, contains 60–70% methane (CH_4), 30–40% carbon dioxide (CO_2), and traces of hydrogen sulfide (H_2S), and hydrogen (H_2).[8] Biogas is an excellent renewable energy source which can be directly used for cooking, lighting, powering a generator to produce electricity and powering motor vehicles.[9]

Most of the livestock manure generated in the world is by cattle and causes environmental problems.[10,11] Cattles are ruminating animals and have methanogenic microbes (belonging to domain archaea) which utilizes hydrogen for production of methane and carbon dioxide in the rumen.[12] Due to the pre-fermentation process in the rumen, cattle manure has been observed to be the most suitable substrate for biogas production over the years.[13] It is rich in proteins and consequently has high nitrogen content. It also has high biological oxygen demand, high organic content but low C/N ratio.[14] Cattle manure contains high water and buffer capacity which has a positive impact on the AD process, however, the leftover lignin from the fodder are resistant to digestion and causes lower biogas production.[10] This issue can be overcome by co-digestion.

Co-digestion is the process of digesting two or more organic substrates with complementary characteristics.[15] It has been proven to enhance the biogas yield by establishing positive synergism in the digestion medium and supply missing nutrients from a single substrate. It also helps to establish the optimal moisture content during the AD process.[16] Co-digesting substrates enables efficient utilization of nutrients and distribution of microbial diversity which can balance the C/N ratio, improve buffer capacity and dilute inhibitors[17–20] and furthermore, it is economically sound choice as the equipment is shared.[21] Anaerobic

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co-digestion of cattle manure has been studied by several researchers, as it carries rich micronutrients necessary for optimal bacterial growth and a high nitrogen content and high buffering capacity which are ideal for enhancing the lignocellulosic biomass digestion.[22] When cattle manure was co-digested with food waste such as tomato residues, the total biogas production was 0.5 to 10.2-fold higher than mono digestion of cattle manure.[23] Co-digestion of cattle manure and barley showed a CH_4 yield of 193.0–230.0 CH_4/g volatile solids (VS), while mono digestion of cattle manure under the same environmental conditions showed a yield of 149 CH_4/g VS thus proving that co-digestion is a favorable approach for biomethane production.[24] Li et al. 2015 reported that CH_4 production yield in co-digestion of cattle manure and rice straw was 196.03 L/kg VS, which was slightly higher than that of mono digestion of cattle manure (185.26 L/kg VS).[25]

Corn stover is a readily available source of biomass for biofuel production. But it has a relatively refractory structure, i.e. crystals of cellulose and a strong association between cellulose and hemicellulose with lignin, which leads to low susceptibility of lignocellulose to hydrolysis.[26] Pretreatment prior to AD is considered an effective method to improve the digestibility of corn stover[27–29] and reduce ammonia concentration as well.[30] Recently, biological pretreatment has become a more attractive approach to enhance the bioconversion of lignocellulose, due to its advantages including low chemical and energy usage, environmental friendliness, minimized the carbohydrate loss, maximized lignin removal and increased digestibility of feedstock.[31] Biological pretreatment of biomass can be carried out by fungi, enzymes or microbial consortium. One of the crucial steps during biological pretreatment for biogas production is combing the right substrate composition with the right pretreatment technique to enhance the bioavailability of the substrate.[32,33] Mackulak et al. used a wood decaying fungus to pre-treat sweet chestnut leaves and hay for 4–5 week and a biogas increase of 15% was observed on comparison with the untreated leaves.[34] Another study reported a significant increase of 154% in CH_4 yield when yard trimmings were pretreated by *C. subvermispora* ATCC 96608 fungus for 30 days.[35] During the enzymatic pretreatment process, enzymes with hydrolytic activity may be applied before or during the AD process. Though the effect of enzyme on the biogas production was minimal, it was reported that when pulp and paper sludge was pretreated with mushroom compost extract, laccase and carboxymethylcellulose a CH_4 yield increase of 34.2% was noticed.[36]

Contrary to fungal pretreatment and enzyme digestion which attack specific substrates, degrading cellulosic biomass with microbial consortium has been proposed as a highly efficient approach for biotechnological applications, because it avoids the problems of feedback regulation and metabolite repression posed by isolated single strains.[37] In order to biologically pretreated cassava residue, thermophilic microbial consortium was mixed with distillery wastewater and added to cassava residue at 55 °C for 12 h, and a 96% increase in CH_4 yield was recorded when compared with the untreated residue.[38] The liquid fraction of digestate (LFD) from anaerobic digester contains abundant microbes necessary for AD process and organic substances such as amino acids, sugars, proteins and inorganic substances such as NH_4^+-N, NO_3-N and potassium ions.[39] The use of LFD as a microbial agent for pretreatment may combine biological and chemical pretreatment to act together on the lignocellulose and offer advantages by reducing the cost and the pollution potential caused by LFD.[27] On comparison with the untreated corn stover, the CH_4 yield showed an increase of 66.30% while the biogas production was enhanced by 70.40% when corn stover was pretreated with LFD under mesophilic conditions.[27]

Temperature is one of the most important aspects of AD.[40] Thermophilic temperature often results in better degradation of lignocellulosic biomass, thus improving AD efficiency and eradicates pathogens as compared to mesophilic AD.[41] To the best of our knowledge, this is the first study on the pre-digestion (i.e. anaerobic-biological pretreatment) and anaerobic co-digestion of corn stover at the thermophilic temperature. This study has two main objectives: (a) investigating the effect of thermophilic pre-digestion of corn stover with LFD and (b) studying the effect of thermophilic anaerobic co-digestion of pre-digested and untreated corn stover and cattle manure on biomethane production.

Materials and methods

Feedstock and characterization

Corn stover was harvested from the North Carolina Agricultural and Technical State University (Greensboro, NC, USA) farm in August 2017. It was later dried at 105 °C in an Isotemp oven for a minimum of 24 h till a constant weight was achieved. The dried corn stover was ground to 0.2–0.5 mm particle size using a Thomas Model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA), and stored at the room temperature.

The liquid digestate was collected from an anaerobic digester running at the North Carolina Agricultural and Technical State University farm. The anaerobic digester was operated at 55 °C with continuous agitation at 250 rpm. The liquid digestate was aseptically transferred to sterile 50 mL centrifuge tubes. The tubes were centrifuged for 20 min at 4,500 rpm to obtain the supernatant, i.e. LFD, which was used for the pre-digestion process.

Fresh cattle manure was collected from the North Carolina Agricultural and Technical State University dairy farm on the day of the experiment and used on wet basis. Total solid (TS), volatile solid (VS), and compositional analysis of all materials were carried out using the laboratory procedures (LAPs) developed by the National Renewable Energy Laboratory (NREL). The determination of cellulose, hemicellulose and lignin was conducted as per LAP#003 protocol.[42] The elemental analysis was conducted using a PE 2400 II CHNS/O analyzer (Perkin Elmer, Waltham, MA, USA). The characteristics of corn stover, LFD, and cattle manure are mentioned in Table 1.

Table 1. Characteristics of corn stover and liquid fraction of digestate.[a]

Sample name	Corn stover	Liquid fraction of digestate	Cattle manure
TS[b] (wt%)	94.56 ± 3.0	0.20 ± 0.6	17.0 ± 2.5
VS[c] (wt%, dry basis)	93.90 ± 0.7	58.5 ± 1.0	85.23 ± 1.7
Cellulose %	34.81 ± 0.72	NA	26.6 ± 0.24
Hemicellulose %	26.95 ± 0.77	NA	18.8 ± 0.98
Lignin %	16.03 ± 0.26	NA	14.2 ± 0.34
Carbon %	47.36 ± 0.7	66.25 ± 6.0	42.58 ± 0.2
Nitrogen %	0.57 ± 0.01	2.17 ± 0.2	2.48 ± 0.04
C/N ratio[d]	83.09	30.53	17.20

[a]Values are means ± standard deviation ($n = 3$).
[b]TS = Total solid.
[c]VS = Volatile solids.
[d]C/N = Carbon-to-nitrogen ratio.

Pre-digestion of corn stover with liquid fraction of digestate

The pre-digestion process was carried out under different conditions including corn stover to LFD ratio, temperature and time duration (as shown in Table 2). Prior to pre-digestion, corn stover was stored in an oven at 105 °C for 24 h to avoid any contaminants or microorganism. All glassware was autoclaved and sterilized at 121 °C for 30 min. For all pre-digestion experiments, the corn stover weight remained constant. All the experiments were conducted in the Automatic Methane Potential Test System (AMPTS II) (Bioprocess Control, Sweden). The LFD and corn stover were transferred aseptically to the 500 mL experiments flasks. All experiments were carried out under anaerobic conditions.

In batch 1–4 (Table 2), the corn stover to LFD ratio was maintained at 1:7, 1:10, 1:13 and 1:16 at 55 °C for 7 days. Corn stover was pre-digested with LFD in the ratio of 1:10 at two different temperatures of 55 °C (batch 5 in Table 2) and 60 °C (batch 6 in Table 2) for a period of 7 days. In batches 7, 8 and 9 (Table 2), the corn stover to LFD ratio was 1:10 and were carried out for a period of 3, 7 and 14 days, respectively at 55 °C.

Each experiment was carried in triplicate. Untreated corn stover was considered as the control. At the end of pre-digestion experiments, the samples were dried in an oven at 105 °C for a minimum of 24 h for compositional analysis. Buffer was not added to any pre-digestion batches.

Anaerobic co-digestion

Based on the results of the pre-digestion experiments, following two conditions were selected to study the effect of pre-digested corn stover on CH_4 production of AD – Corn stover pre-digested with LFD (1:10) at 55 °C for 3 days (3-day-CS) and corn stover pre-digested with LFD (1:10) at 55 °C for 7 days (7-day-CS). The selected batches were dried at 105 °C in an Isotemp oven for a minimum of 24 h.

All AD experiments were conducted in the AMPTS II unit. The AMPTS II unit is an analytical device for biomethane potential, which accurately measures and records the biomethane produced from the feedstock.[43] Anaerobic co-digestion experiments were carried out with dried pre-digested corn stover, cattle manure, and liquid digestate. For each co-digestion experiment, various amount of pre-digested corn stover (dry weight), 50 g cattle manure (wet weight) and 200 g liquid digestate were placed into 500 mL flasks with a working volume of 400 mL. Water was added to adjust the TS content to ~4%. The pH value was adjusted to 7.10 as it is the optimum pH[25] and 0.1 M potassium phosphate buffer was used to adjust the pH.[44]

All anaerobic co-digestion experiments were carried out in triplicate at 55 °C for a total period of 21 days. On day 3, 7, 14 and 21, the biomethane yield and biogas composition were recorded, and samples were drawn to evaluate pH and VS. The biogas composition was analyzed using Biogas 5000 analyzer (Landtech North America, Dexter, MI, USA). AD of cattle manure was used as the control.

Results and discussion

Pre-digestion of corn stover

The main purpose of pre-digestion is to facilitate production of more biodigestible cellulose and hemicellulose. The changes in mass reduction and the chemical composition of corn stover after pre-digestion are presented in Table 2.

In batch 1–4, the corn stover to LFD ratio was maintained at 1:7, 1:10, 1:13 and 1:16 at 55 °C for a period of 7 days. The reduction of lignin, cellulose and hemicellulose (LCH) content was higher as the ratio of LFD increased. The cellulose content reduced by 13.10%, 27.93%, 34.21% and 52.62% for the corn stover to LFD ratios of 1:7, 1:10, 1:13 and 1:16, respectively, compared to the undigested corn stover. It was observed that the ratios of lignin and hemicellulose decomposed more than that of cellulose. This could be due to either solubilization or conversion of some quantity of lignocellulose into other components.

The pre-digestion batch 5 at 55 °C and batch 6 at 60 °C, statistically did not show any significant difference. However, greater reduction in lignin was observed as compared to cellulose and hemicellulose. A possible explanation could be that higher temperature liberates acid and facilitates the breakdown of ether linkages in biomass.[45]

In batches 7–9, the pre-digestion was performed over a period of 14 days. The contents of cellulose, hemicellulose and lignin were reduced by 36.90%, 40.10% and 41.01%, respectively. The results indicated that longer the corn stover remained in contact with the LFD, more was the cellulose and hemicellulose reduction. The changes in the chemical composition could be attributed to microbial population in the LFD.

It was observed in all the batches that the LCH content was lower in the pre-digested corn stover as compared to the untreated corn stover. The decrease in LCH content can be associated to the enzymatic activity of microorganisms. According to Zhong et al. 2011,[2] the biodegradability potential can also be estimated by the ratio of lignin to cellulose. The lignin to cellulose ratio of the pre-digested corn stover was lower than the undigested corn stover justifying the enhancement of biodegradability of pre-digested corn stover by microbial agents.[2]

Table 2. Changes in the cellulose, hemicellulose, and lignin percentage at different ratios of corn stover to liquid fraction of digestate.[a]

Batch no.	Corn stover to LFD ratio	Temperature	No. of days	Composition analysis: Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	Total LCH[b] (%)	Lignin/cellulose	Mass reduction ratio: Total mass reduction (%)	Cellulose reduction (%)	Hemicellulose reduction (%)	Lignin reduction (%)
–	Undigested	–	–	34.81 ± 0.72	26.95 ± 0.77	16.03 ± 0.26	77.78	0.460	NA	NA	NA	NA
1	1:7	55	7	34.76 ± 0.05	25.21 ± 0.25	14.73 ± 0.19	74.69	0.424	13.00 ± 2.27	13.06 ± 2.92	18.55 ± 2.73	19.84 ± 2.69
2	1:10	55	7	33.60 ± 0.43	24.46 ± 0.36	14.12 ± 0.57	72.18	0.420	25.33 ± 7.67	27.93 ± 7.40	32.27 ± 6.96	34.11 ± 6.77
3	1:13	55	7	33.10 ± 0.54	23.29 ± 0.68	13.72 ± 0.35	70.11	0.415	30.67 ± 2.32	34.21 ± 2.21	40.21 ± 2.01	40.67 ± 1.99
4	1:16	55	7	32.70 ± 1.31	22.11 ± 0.28	13.64 ± 0.28	68.46	0.417	49.57 ± 6.41	52.62 ± 6.02	58.62 ± 5.26	57.01 ± 5.57
5	1:10	55	7	33.60 ± 0.43	24.46 ± 0.36	14.12 ± 0.57	72.18	0.420	25.33 ± 7.67	27.93 ± 7.40	32.27 ± 6.96	34.11 ± 6.77
6	1:10	60	7	33.44 ± 0.19	23.51 ± 0.61	12.75 ± 1.21	69.70	0.381	25.3 ± 5.47	27.61 ± 2.83	34.57 ± 6.94	40.23 ± 7.21
7	1:10	55	3	34.10 ± 1.20	24.73 ± 0.32	14.41 ± 0.14	73.24	0.423	16.63 ± 2.46	18.32 ± 4.92	23.48 ± 4.16	24.92 ± 3.76
8	1:10	55	7	33.60 ± 0.43	24.46 ± 0.36	14.12 ± 0.57	72.18	0.420	25.33 ± 7.67	27.93 ± 7.40	32.27 ± 6.96	34.11 ± 6.77
9	1:10	55	14	31.70 ± 0.15	23.31 ± 0.46	13.62 ± 0.04	68.63	0.416	31.00 ± 3.63	36.96 ± 2.02	40.08 ± 4.65	41.09 ± 2.68

[a]Values are means ± standard deviation ($n = 3$).

The pH change during pre-digestion

LFD used for the pre-digestion experiment was at neutral pH and hence no additional buffer was added to any of the pre-digestion batches. Throughout the pre-digestion experiments, no methane gas was produced and the H_2S level was always above 5000 ppm. It was observed that there was a substantial decrease in pH on day 3 (5.44) which continued to drop to 4.95 on day 7 and finally showed a minor increase to 5.14 on day 21. The acidic environment inhibited the methane production. A possible explanation for the pH drop could be attributed to H_2S production and volatile fatty acid (VFA) accumulation. AD of organic waste is heavily dependent on the acid producing and methane producing rate. If the acidic producing process is faster, VFA accumulate occurs which tends to reduce pH and in turn inhibits the growth of methanogens due to the loss of acid-sensitive glycolytic enzymes.[46] The problem of VFA accumulation or pH drop can be reduced by the co-digestion process.[17] Also, the lack in buffer could have led to unstable pH values.

The requirement of large quantities of LFD or longer pre-digestion time durations could lead to substantial loss in cellulose and hemicellulose[47] and increase costs and contaminations.[48] Therefore, only corn stover pre-digested with LFD (1:10) at 55 °C for 3 days and 7 days was selected as the feedstock for anaerobic co-digestion tests.

Anaerobic co-digestion

Anaerobic co-digestion of corn stover with cattle manure

At the end of pre-digestion experiments, the selected pre-digested corn stover batches were dried at 105 °C in an Isotemp oven for a minimum of 24 h. Both untreated corn stover and corn stover pre-digested for 3 days and 7 days (i.e., 3-day-CS and 7-day-CS) were anaerobically co-digested with cattle manure for 21 days at 50 and 55 °C to study the CH_4 production. However, almost all of AD tests conducted at 50 °C were contaminated with fungi within 3–5 days of the start of the experiments, resulting no methane production. This is most likely due to the use of fresh cattle manure. By further rising the AD temperature, the contamination problem was solved and biomethane production was favorable at 55 °C. The CH_4 yield of the untreated and pre-digested corn stover batches are shown in Figure 1.

For all the batches, the CH_4 yield showed a stable increase from day 3 to day 21 with the highest CH_4 yield observed on day 21. The CH_4 yield of 3-day-CS was 193.35, 314.23 and 413.48 mL/g VS on day 3, 7 and 14, respectively. The co-digestion batch of 7-day-CS recorded a CH_4 yield of 289.89, 391.01 and 467.16 mL/g VS on day 3, 7 and 14, respectively. On day 21, the control batch (cattle manure only) showed a value of 189.9 mL/g VS. Co-digestion of untreated corn stover and cattle manure resulted in a CH_4 yield of 357.41 mL/g VS, while co-digestion of 3-day-CS and 7-day-CS yielded 446.84 mL/g VS and 518.58 mL/g VS, respectively.

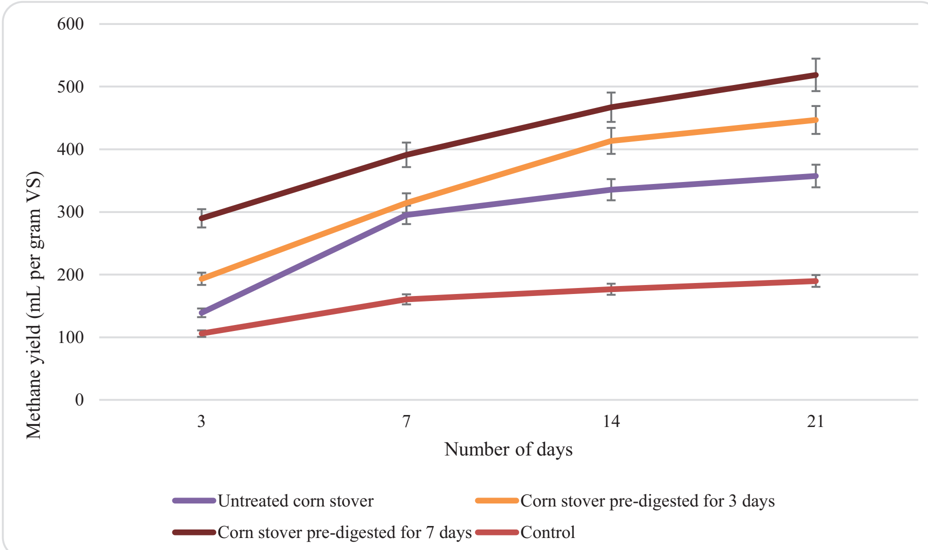

Figure 1. Methane production of undigested and pre-digested corn stover.

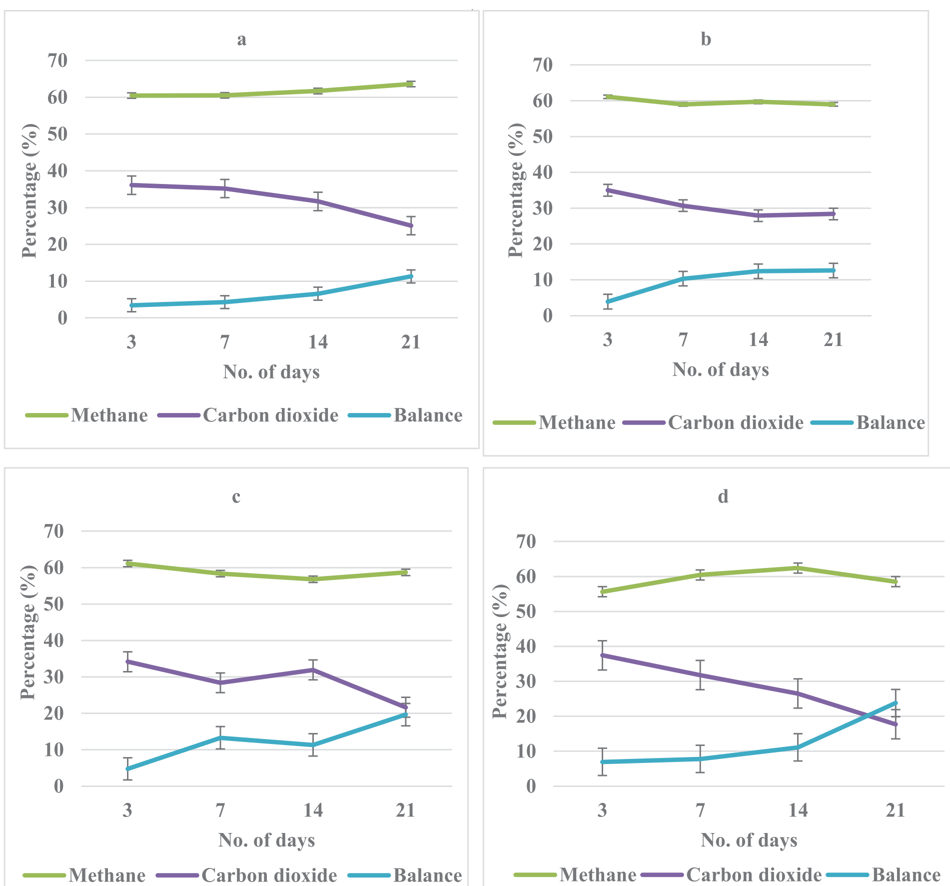

Figure 2. Composition of biogas produced by anaerobic co-digestion of (a) untreated corn stover, (b) corn stover pre-digested for 3 days, (c) corn stover pre-digested for 7 days, and (d)Control.

It was observed that the biomethane production increased when corn stover was pre-digested for longer time duration. A CH_4 yield increase of 45.09% was observed when corn stover was pre-digested for 7 days with LFD. The criteria for determining the success of co-digestion are VS and CH_4 yield.[49] A higher biomethane production value from pre-digested corn stover could be attributed to the increase in the amount of digestible cellulose and hemicellulose after the pre-digestion step.

Biogas composition

The co-digestion tests of all feedstocks produced a fair amount of biogas that consists of methane, carbon dioxide

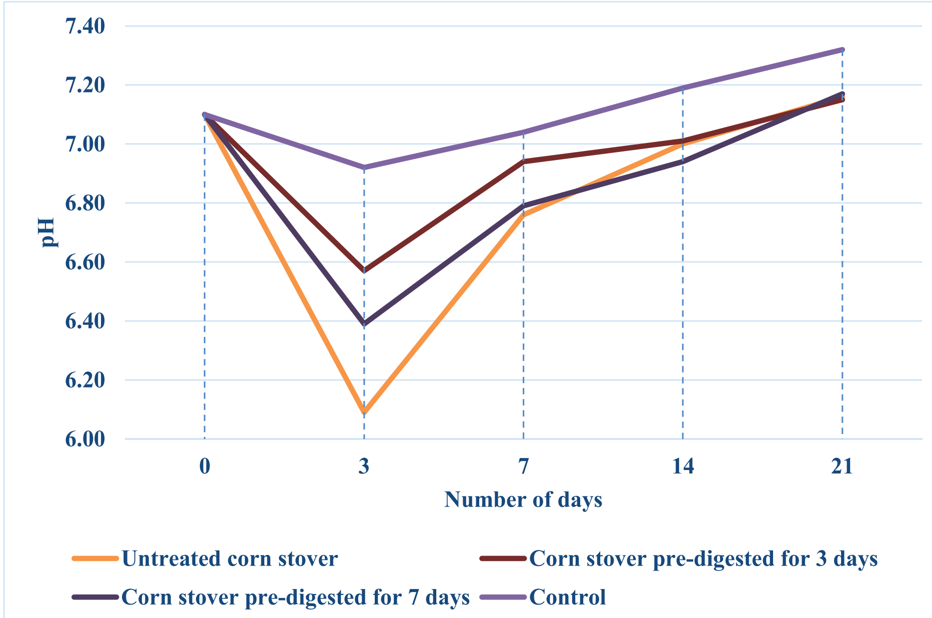

Figure 3. Changes in pH values during anaerobic co-digestion.

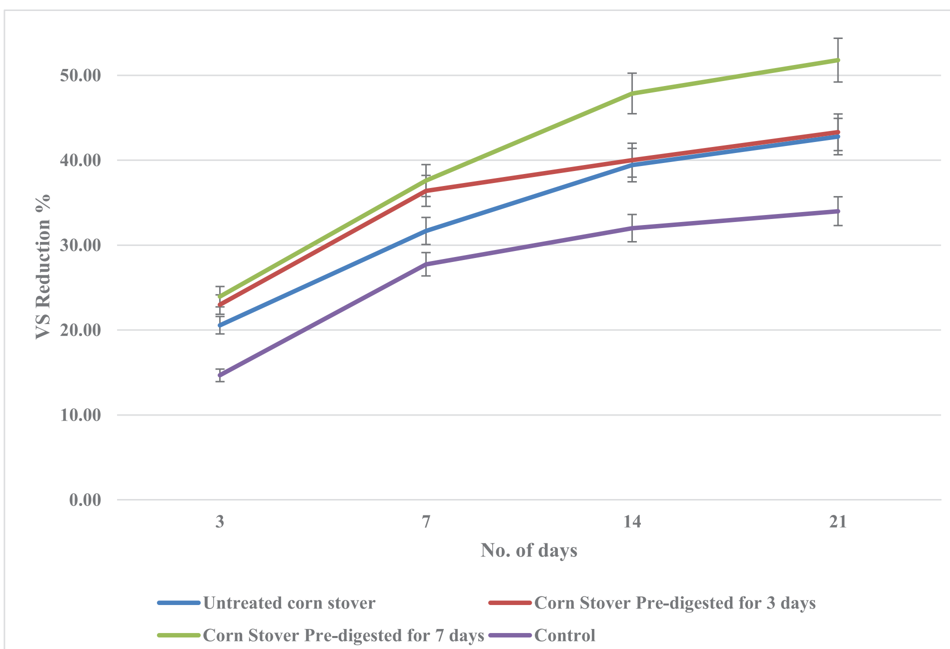

Figure 4. Volatile solids reduction rate during the AD process.

and traces of H_2S. The CH_4 content of biogas produced was around 60 vol% (Fig. 2), while the CO_2 content was between 18 and 38 vol%. At the end of anaerobic co-digestion (day 21), the CO_2 content reached the lowest level, indicating the presence of increasing oxygen and nitrogen gas while the CH_4 content was stable.

Change in pH

For all anaerobic co-digestion experiments, the initial pH was adjusted to 7.1 as this pH is optimal for the growth of methanogenic microorganisms. The pH value was not further controlled during the AD process. A pH decrease was noticed on day 3 for all batches. This suggested the presence of fatty acids caused by hydrolytic bacteria which breakdown complex organic matters into sugars, amino acids and fatty acids. The control batch (i.e., cattle manure only) demonstrated the least change in the pH value throughout the AD process. The untreated corn stover exhibited the lowest pH value of 6.09 on day 3, while 3-day-CS and 7-day-CS showed pH values of 6.57 and 6.39, respectively. A possible explanation would be the presence of more undigested organic matter in the untreated corn stover which could have led to a higher number of fatty acid production.[50]

From day 3 to 7, an increase in pH was observed in all batches. The pH value of the untreated corn stover co-digestion batch increased to 6.76, while 3-day-CS and 7-day-CS had pH values of 6.94 and 6.79, respectively. This could be explained by the action of acidogenic bacteria which utilize the compounds formed during hydrolysis of fatty acids and are further digested into VFA along with ammonia, H_2S, CO_2 and H_2. As per previous studies, the changes in pH during acidogenesis phase ranged from 5.0 to 6.5.[50] The bacteria in the hydrolysis phase have the ability to withstand the environmental condition perturbance without any loss of activity.[51] On day 14, the pH values of co-digestion of untreated corn stover, 3-day-CS, 7-day-CS and control were 7.00, 7.01, 6.94 and 7.19, respectively. The pH increase may be due to acetogenesis, in which higher organic acids and alcohols are produced by acidogens to produce mainly acetic acid along with CO_2 and H_2.[52] On day 21, all co-digestion batches had a pH value close to 7.10, while the control had a slightly higher pH of 7.32 (Fig. 3).

Biodegradability improvement

The VS reduction rate is a crucial parameter to measure the biodegradability in AD.[53] The reduction of substrates and the increase in CH_4 yield with respect to VS were used to evaluate the digestion performance of untreated and pre-digested corn stover. On day 3 of AD, the VS reduction for untreated corn stover, 3-day-CS and 7-day-CS was 20.56%, 23.00% and 23.93%, respectively. On day 21, the VS conversion efficiencies for the co-digestion of cattle manure with untreated corn stover, 3-day-CS and 7-day-CS were 42.78%, 43.30% and 51.79%, respectively, which were higher than that of cattle manure alone (34.00%) (Fig. 4). On comparison with the CH_4 yield in Figure 2, it was indicated that that higher VS reduction rate led to higher biomethane production. The increase of biogas production can be attributed to higher biodegradability because of pre-digestion of corn stover with LFD. This could be due to the synergistic effect of complex microbial agents in the liquid digestate. It was observed that the components were more available to anaerobic microorganisms after pre-digestion process, representing significant improvement in biodegradability. Hence, the two-stage anaerobic co-digestion demonstrated a positive impact on the methane yield.

Conclusion

Thermophilic pre-digestion of corn stover with LFD proved to be an efficient method to enhance the biodegradability of corn stover. In the first pre-digestion batch, when corn stover was pre-digested with LFD at 1:16 ratio for 7 days, the LCH reduction of 11.98% was recorded as the highest; in the second pre-digestion batch, the highest LCH reduction was 10.39% at 60 °C for 7 days and for the third batch, the highest LCH reduction of 11.76% was observed on day 14 of pre-digestion when compared to untreated corn stover. The decrease in the LCH is an indicator that LFD treatment has the ability to destroy the lignocellulosic structure. At the end of pre-digestion, the digested corn stover was anaerobically co-digested with cattle manure at 55 °C for 21 days. AD of corn stover pre-digested for 3 days and 7 days yielded methane at a value of 446.84 mL/g VS and 518.58 mL/g VS, respectively, thus establishing that pre-digestion time played a crucial role in biodegradability of corn stover. The changes in the composition of the pre-digested corn stover had a positive effect on the biodegradability and contributed to an increase in biomethane production, thus highlighting that the performance of two-stage anaerobic co-digestion was better. AD of organic wastes, thus, provides an efficient method for waste treatment to reduce the harmful effect of waste on the environment and recover energy, nutrients and water which can be recycled and reused for sustainable agricultural production.

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