Integrated Methane and Ethanol Production from Livestock Manure and Soybean Straw

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Methane and ethanol were co-produced from different feedstock, including a mixture of dairy manure and soybean straw (DMS), a mixture of pig manure and soybean straw (PMS), and soybean straw alone (SS), after anaerobic digestion times of 30 and 60 days in mesophilic conditions. Digesting DMS for 60 days led to the highest methane yield of 115.3 g/kg dry raw feed; however, the lowest ethanol yield of 88 g/kg dry raw feed was observed. After 30 days, SS yielded the lowest methane levels (45.2 g/kg dry raw feed) but the highest ethanol levels (113.5 g/kg dry raw feed). Analysis of the net energy balance showed that the highest net energy balance, 6549 kJ/kg of dry raw feedstock, was achieved from the digestion of DMS for 60 days. Overall, both the type of feedstock and length of digestion time played important roles in the integrated processing of methane and ethanol from livestock manure and straw.

Keywords: Soybean straw; Livestock manures; Anaerobic digestion; Ethanol fermentation; Pretreatment

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INTRODUCTION

Concerns have been growing about potential energy crises and environmental pollution. Therefore, the production of biogas, ethanol, and other bio-chemicals from lignocellulosic biomass has attracted increasing attention as a low-cost method to produce these products from readily available materials (Monlau *et al*. 2015).

However, there is also some residual carbon in digestate from anaerobic digestion (AD process), and this can be used to produce ethanol. Recently published reports have demonstrated the feasibility of such a process (Yue *et al*. 2010; MacLellan *et al*. 2013; Wang *et al*. 2016a). There are drawbacks of using digestate as a fertilizer, which may pose an environmental pollution risk (Gioelli *et al*. 2011; Sambusiti *et al*. 2013; Zirkler *et al*. 2014) and may have high shipping costs (Rehl and Müller 2011; Hoffpauir and Wurbs 2012). In addition, the net energy attained from co-production of methane and ethanol is higher than from the production of ethanol alone (Rabelo *et al*. 2011; Teater *et al*. 2011).

The C/N ratio is an important factor in anaerobic digestion (Wu *et al*. 2010; Ge *et al*. 2016). This ratio should range from 20 to 30 (Yen and Brune 2007; Chen *et al*. 2008). Generally, lignocellulosic biomasses are rich in carbohydrates but poor in nitrogen, *i.e.*, they have high C/N values (Giuliano *et al*. 2013; Ye *et al*. 2013). Therefore, the mono-digestion of lignocellulosic biomass may result in poor methane yields (Sawatdeenarunat *et al*. 2015; Ge *et al*. 2016). In contrast, livestock manure is often rich in nitrogen but lacks carbohydrates, *i.e.*, they have low C/N values.

The mono-digestion of livestock manure also results in low methane production due to the inhibition of anaerobic digestion by the ammonia byproduct produced from organic nitrogen decomposition (Abouelenien *et al*. 2014). Thus, the co-digestion of lignocellulosic biomass and livestock manure should balance the C/N ratio and improve methane production.

Nitrogen is a crucial nutrient in ethanol fermentation, which controls the fermentation capacity and affects capital costs (Ma *et al*. 2016). Yeast extract, peptone, urea, and ammonium sulfate are used as nitrogen sources during ethanol fermentation, which increases the total cost of cellulosic ethanol production (Ma *et al*. 2016). Importantly, there is some available residual nitrogen in digestate that can be used as a nitrogen source for ethanol fermentation. Thus, using solid digestate as feedstock for ethanol production could fully utilize agricultural wastes, improve the ethanol fermentation efficiency, and reduce the cost of nitrogen sources in bioethanol production.

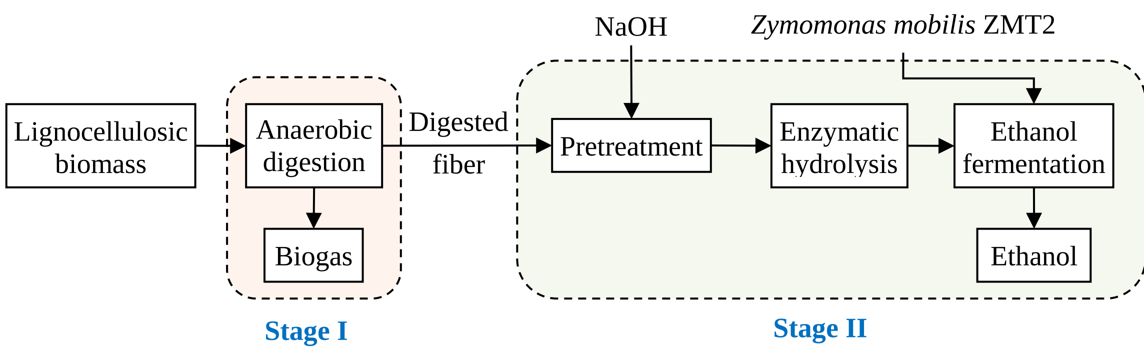

Fig. 1. Schematic diagram of the integrated methane and ethanol production

While several studies have been published related to the utilization of digestate for bioethanol production, none have considered soybean straw as a feedstock. The aim of this study was to investigate the potential of co-production of methane and ethanol from soybean straw and livestock manure. A schematic diagram of the envisioned process is shown in Fig. 1. Stage I was for biogas production, and stage II was for ethanol production. Different feedstock compositions and digestion times were investigated to determine the optimal conditions for integrating these processes. In addition, mass and energy balances were calculated to provide further insight into this production strategy.

EXPERIMENTAL

Materials

Raw dairy manure, pig manure, and soybean straw were collected from a local farm in the suburb of Chengdu, China. The soybean straw was air-dried, shredded, and ground into small pieces. The characteristics of the feedstocks are presented in Table 1. The C/N ratios in the dairy manure, pig manure, and soybean straw were 23, 31.4, and 30.2, respectively.

Table 1. Feedstock Characteristics

Component	Dairy Manure	Pig Manure	Soybean Straw
Total solids (%)	18.9 ± 0.08	28.4 ± 0.46	92.8 ± 0.36
Volatile solids (% TS)	78.3 ± 0.93	83.1 ± 0.59	92.2 ± 0.22
Cellulose (% TS)	24.57 ± 1.27	27.01 ± 2.3	31.9 ± 0.56
Hemicellulose (% TS)	22.87 ± 0.26	21.06 ± 0.31	18.08 ± 1.61
Lignin (% TS)	8.95 ± 0.54	15.05 ± 0.03	12.5 ± 1.15
Carbon (% TS)	37.74	40.24	39.87
Nitrogen (% TS)	1.64	1.28	1.32
Hydrogen (% TS)	5.25	5.35	5.90
Sulphur (% TS)	< 0.50	< 0.50	< 0.50

Methods

Anaerobic digestion

After complete premixing, batch fermentations of the three substrates of dairy manure with soybean straw (DMS), pig manure with soybean straw (PMS), and pure soybean straw (SS) were performed. The ratios of soybean straw to dairy or to pig manure were 40:60 in the DMS and PMS feedstock, with the best production from the co-digestion of corn stover and swine manure (MacLellan *et al*. 2013). A 2.5 L glass bottle with a working volume of 1.8 L was used as digester for this test. The bottle was sealed using rubber plugs with two pipes. One pipe was used for extracting the biogas, and the other pipe was connected to the bottle filled with water. At the start of the anaerobic digestion process, the reactors were purged with nitrogen gas for about 4 min to ensure anaerobic conditions. Reactions were conducted under mesophilic (35 ºC) conditions using water bath for 30 days and 60 days, separately. The digested sludge from a pig farm was used as inoculum. The ratio of feedstock to inoculum in each reactor was 1:1 (TS %). The total solid content (TS) of each reactor was 20%. The biogas production was measured using a water displacement method (see below).

Dilute alkali pretreatment

Solid digestates (AD fibers) containing 5% TS (total solid) were pretreated with 2% sodium hydroxide at 130 ºC for 2 h prior to enzymatic hydrolysis. The treated samples were separated into liquid and solid residues. The liquid samples were used for high performance liquid chromatography (HPLC) to quantify glucose and xylose, while the solid samples were dried and processed for enzymatic hydrolysis.

Enzymatic hydrolysis

Alkali-pretreated samples (0.3 g dry matter) were mixed with 30 mL of sodium citrate buffer (50 mM; pH 4.8) in a 125 mL shake flask, resulting in a 1% concentration of solid (m/v). Cellulase (50 μL, Sigma Co., Denmark) from *Trichoderma reesei* ATCC 26921 contained 700 units/g was added into each flask. The flask was then incubated at 50 ºC with shaking at 150 rpm for 72 h; afterwards, the flask was then heated to 100 ºC for 10 min to inactivate the enzymes. HPLC was used to quantify glucose and xylose in the liquid samples.

Ethanol fermentation

Zymomonas mobilis ZMT2 (CGMCC11888, our lab, stored at China General Microbiological Culture Collection Center) (Wang *et al.* 2016b) was cultured on a rich medium at 30 ºC without shaking (Goodman *et al*. 1982), and maintained on agar fortified with glucose (20.0 g/L glucose, 10.0 g/L yeast extract, and 15.0 g/L agar). For fermentation, the *Z. mobilis* was sub-cultured onto fresh inoculum media for 24 h at 30 ºC, and then a 10% (v/v) inoculum was transferred into the fermentation medium to get an OD_{600} of 0.35. Inoculum medium (g/L) consisted of 10.0 g yeast extract, 1.0 g $MgCl_2$, 1.0 g $(NH_4)_2SO_4$, 1.0 g KH_2PO_4, and 20.0 g glucose. After fermenting for 24 h, the samples were removed, filtered, and analyzed by HPLC to quantify the glucose and ethanol.

Analysis methods

Biogas production was measured by water displacement, and biogas composition was quantified by gas chromatography (GC122, Shanghai Instrument-electric Analysis Instrument Co., Ltd., Shanghai, China) equipped with a thermal conductivity detector (TCD) (Wang, *et al*. 2016a). The stainless steel column used was packed with Porapak Q. Injector, oven, and detector temperatures were 120, 120, and 150 ºC, respectively. Nitrogen was used as a carrier gas and flow was maintained at 30 mL min^{-1}. Total solids (TS) and volatile solids (VS) were measured according to standard methods (APHA 2012). Lignocellulose was measured with a cellulose analyzer (FOSS Fibertec™ 2010, FOSS, USA), while carbon and nitrogen were measured using a Vario MICRO select elemental analyzer (Elementar, Mt. Laurel, NJ, USA). Glucose, xylose, and ethanol were quantified using High Performance Liquid Chromatography (HPLC, Agilent Technologies, Palo alto, California, USA). HPX-87H ion exclusion column (BioRadAminex) was used with a sulfuric acid solution (0.05 M) as the mobile phase at a flow rate of 0.6 mL/min and a column temperature of 35 ºC.

Analysis of net energy balance

The energy input into AD was calculated using the method of MacLellan *et al*. (2013). The energy input into ethanol fermentation was calculated by the method of Piccolo and Bezzo (2009), and the energy output of AD and ethanol fermentation were calculated by the method of Wang *et al*. (2016a).

RESULTS AND DISCUSSION

Performance of anaerobic digestion

The average amount of biogas produced per gram VS per day and the cumulative methane produced from soybean straw and livestock manure after 30 days of AD are shown in Figs. 2a and 3a, respectively. After 30 days, AD of the DMS substrate resulted in the most biogas produced (3.4 mL/g VS per·day), which was followed by PMS (1.5 mL/g VS per·day). When using SS, both the initial and average amounts of biogas generated were at their lowest levels of all the conditions tested (0.04 mL/g VS per·day). This was due to an accumulation of volatile fatty acids, which acidified the medium and inhibited biogas production (Chen *et al*. 2015). The SS after 30 days had a pH of 5.46, which was lower than the pH of the DMS (7.84) and PMS (6.85) after 30 days. Methanogenic bacteria are very sensitive to acidic conditions, and their growth and methane production are inhibited in an acidic environment. The ideal pH for methanogens ranges from 6.8 to 7.2, and their growth rate is significantly reduced when the pH drops below 6.6 (Mosey and Fernandes 1989). Specifically, a pH below 6.1 or above 8.3 has been reported by Lay *et al.* (1997) to cause poor digester performance or digester failure.

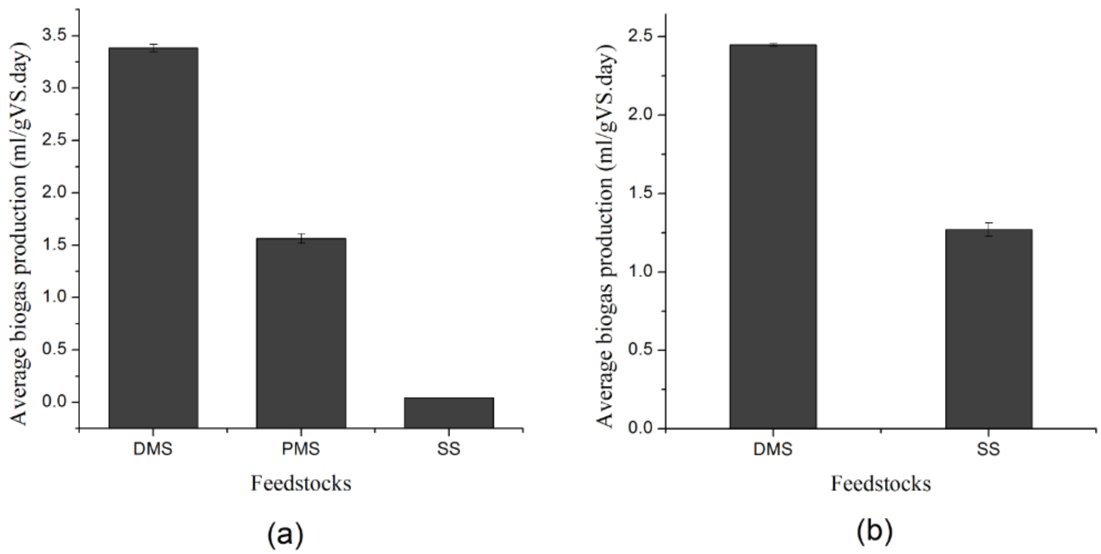

Fig. 2. Average biogas production per day from anaerobic digestion after (a) 30 and (b) 60 days

In terms of cumulative methane production, DMS also performed the best, generating up to 43.9 mL/g VS. In contrast, the cumulative methane produced from PMS was almost two times lower than DMS, likely due to the comparatively later start-up process. Notably, there was almost no methane produced from SS after 30 days, which was attributed to the more rigid lignocellulosic structure of soybean straw (Yang *et al*. 2015) and higher C/N ratio (36.77). The useable C/N ratio falls within a range of 20 to 30, and the optimal C/N ratio for dry AD is 25 (Yen and Brune 2007).

The average amount of biogas produced (Fig. 2b) and methane accumulated (Fig. 3b) from DMS and SS after 60 days were also investigated. The average amount of biogas produced from DMS after 60 days was 2.4 mL/g VS per day, which was less than that the daily average amount after 30 days (3.4 mL/g VS per day). This result indicated that biogas production from DMS decreased as the length of AD increased from 30 to 60 days. The greatest increase in biogas accumulation occurred after shifting from a 30 to 60 days of digestion time for SS.

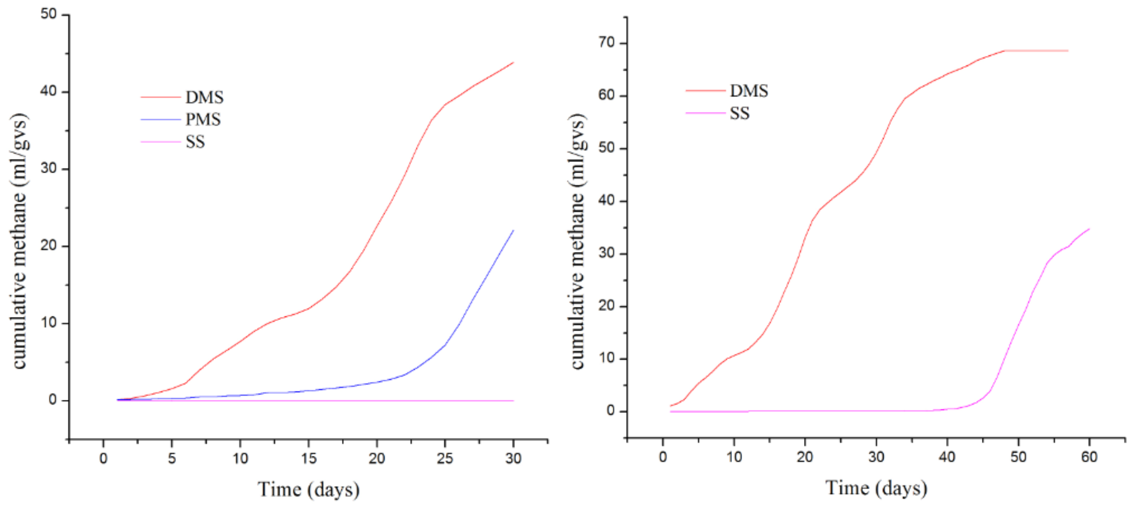

Fig. 3. Cumulative methane production from anaerobic digestion after (a) 30 and (b) 60 days

Following 60 days of digestion, 1.3 mL/g VS per day biogas was generated, which was much higher than after 30 days of digestion. This difference was due to slower start-up process of the SS compared to DMS. These results suggest that increasing the digestion time resulted in higher amounts of biodegradable carbon converted into methane. In contrast, the amount of available carbon in the DMS decreased over time as degradation occurred during AD, which may have caused poor digester performance or even digester failure. Generally, the DMS performed better in terms of total biogas produced after 60 days than SS, although the SS displayed a higher rate in biogas production from 30 to 60 days. As the digestion time increased, the pH of the feedstock increased, especially for SS, which shifted from 5.76 at 30 days to 7.68 at 60 days. These results were consistent with a report that showed an increase in ammonia concentration and pH as digestion reached the methanogenesis stage (Mata-Alvarez *et al.* 2000); this may explain why SS produced remarkably more biogas after 60 days than 30 days.

Nitrogen contents of solid digestates

The residual nitrogen in solid digestates from different feedstocks and after different digestion times was investigated. The nitrogen contents in solid digestate from DMS and SS after 30 days were 1.62% and 1.06%, respectively. The solid digestate from DMS after 60 days had more nitrogen than SS at 1.61% and 1.45%, respectively. DMS had more nitrogen than SS due to the addition of dairy manure, which contains more nitrogen than soybean straw. As previously mentioned, residual nitrogen in the digestate is a nitrogen source for ethanol fermentation. Overall, the co-digestion of livestock manure and straw was beneficial for concurrent production of methane and ethanol.

Glucose and xylose yields

AD fibers were attained by the separation of digestate into solid and liquid forms. Alkali pretreatment of raw SS was required prior to its enzymatic hydrolysis to achieve a high conversion of carbohydrates into reducing sugars. Specifically, treatment of SS with a dilute alkali made the fibers amenable for subsequent enzymatic hydrolysis by disrupting ester bonds that cross-link the cell wall matrix and hydrolyzing acetyl groups in the digested fiber (Taherzadeh and Karimi 2008; Kumar *et al*. 2009). The glucose and xylose yields were generated under different conditions, including different AD fibers after 30 (Fig. 4a) and 60 (Fig. 4b) days.

The AD fibers obtained from DMS after 30 days had the most glucose (55.1%) but the least xylose (14.1%). In comparison, PMS yielded 45.1% glucose and 15.2% xylose, while SS yielded 23% glucose and 17.5% xylose at the same conditions. These results indicated that AD may exclusively promote methane production from the hemicelluloses while retaining the cellulose and lignin in the solid residue (Yue *et al*. 2011; Sawatdeenarunat *et al*. 2015). Yue *et al*. (2011) demonstrated that AD fibers obtained from digestion of manure contained less hemicelluloses (11%) and more cellulose (32%) than raw manure.

The removal of hemicelluloses can effectively destroy the complex SS biomass structure, thus improving the accessibility of the cellulose by enzymes in the downstream processes (MacLellan *et al*. 2013). In this study, AD produced more biogas from DMS than from other feedstocks, which meant that there was a higher consumption of the hemicellulose in the DMS. There were notably less hemicelluloses and more cellulose following the digestion of DMS than with other feedstocks.

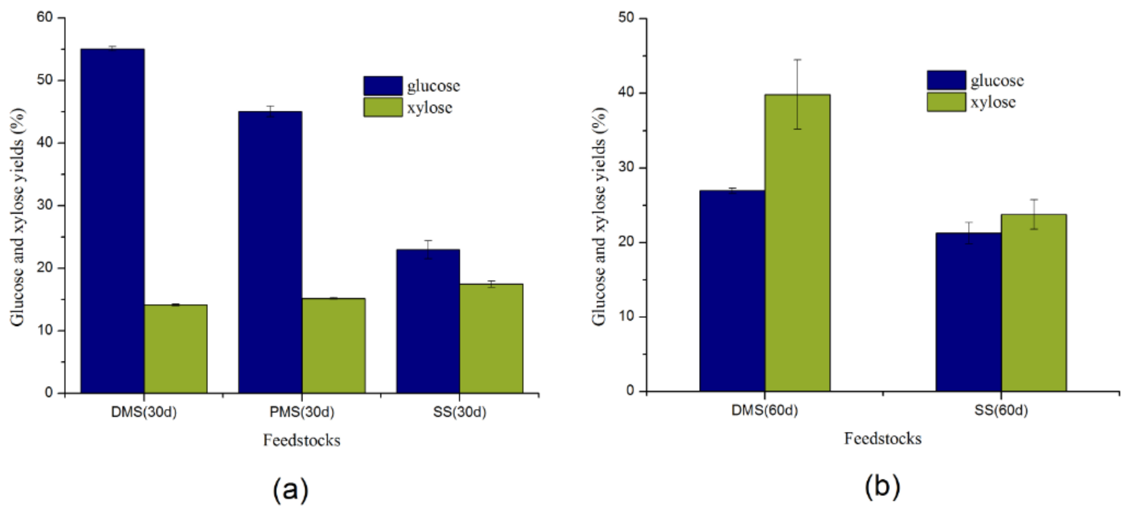

Fig. 4. Glucose and xylose yields from feedstocks after (a) 30 and (b) 60 days

As AD progressed, biogas production from the DMS gradually decreased, which suggested that the removal of the hemicelluloses was inhibited. As shown in Fig. 4b, the comparable yield of glucose after 60 days decreased while that of xylose increased. The AD of SS resembled that of DMS. The glucose and xylose yields after 60 days of AD of the SS fibers were 27% and 39.8%, whereas the AD of the DMS yielded 21.3% and 23.8%, respectively. However, the difference between the glucose and the xylose produced from SS was less dramatic than from DMS. This result was attributed to the different characteristics of the feedstock, especially the C/N ratio, which could significantly influence biogas production and further affect the glucose and xylose yields.

Ethanol production

Ethanol fermentation by *Z. mobilis* ZMT2 was conducted using hydrolysate as substrate. Ethanol concentrations were quantified for several samples, and the results are shown in Table 2.

Table 2. Ethanol Concentration of Pretreated Samples

Feedstock	Ethanol (g/L)
SS (raw)[a]	1.24 ± 0.03
SS (30 days)[b]	1.14 ± 0.04
SS (60 days)[c]	1.09 ± 0.06
SS (60 days un-pretreated)[d]	0.85 ± 0.10
DMS (30 days)[e]	0.98 ± 0.03
PMS (30 days)[f]	0.99 ± 0.04

[a] SS (raw) represents raw soybean straw.
[b] SS (30 days) represents solid digestate attained from soybean straw after 30 days digestion.
[c] SS (60 days) represents solid digestate attained from soybean straw after 60 days digestion.
[d] SS (60 days un-pretreated) represents solid digestate attained from soybean straw after 60 days digestion without NaOH pretreatment.
[e] DMS (30 days) represents solid digestate attained from the mixture of dairy manure and soybean straw after 30 days digestion.
[f] PMS (30 days) represents solid digestate attained from the mixture of pig manure and soybean straw after 30 days digestion.

In accordance with the biogas production results, the ethanol production of AD fibers from SS was the highest, which was likely due to the higher amounts of residual carbon compared with DMS and PMS. Naturally, as the amount of AD time increased, the amount of residual carbon decreased, which resulted in less ethanol production because more carbohydrates became degraded over time (Wang *et al*. 2016a). Therefore, ethanol production from raw SS was higher than from SS after 30 or 60 days of AD. In addition, digestate pretreated with dilute alkali before enzymatic hydrolysis produced more ethanol than the control (untreated), which indicated that the pretreatment was effective. As previously demonstrated, pretreatment of AD fibers with dilute alkali removes a large portion of hemicelluloses (Teater *et al*. 2011).

The overall ethanol production was not higher than in other similar reported studies because the yeast strain could not consume xylose to produce ethanol. Moreover, the substrate concentration was 1% (m/v) for enzymatic hydrolysis in this study, which was lower than in other reports (MacLellan *et al*. 2013; Wang *et al*. 2016a). In future studies, continuous fermentation should be considered to improve ethanol yields.

Mass and energy balances

Mass and energy balances were also calculated based on the methane and ethanol produced (Table 3). This data allows comparisons between the net energy obtained from different feedstocks and digestion times. Based on the published literature conventions, the energy inputs were indicated as negative, while the energy outputs were indicated as positive (MacLellan *et al*. 2013; Wang *et al*. 2016a). When considering the raw materials, DMS was the preferred choice for the co-production of methane and ethanol. The total methane produced from DMS after 30 days and 60 days was 91 g/kg and 115.3 g/kg, respectively. The DMS after 60 days of AD had the highest net energy, despite having the lowest ethanol yield, because integrated methane and ethanol production depend on efficient AD (Wang *et al*. 2016a). For example, the net energy obtained from DMS after

60 days was the highest due to the efficient operation of AD. By contrast, the net energy gained from raw SS after 30 days was the lowest due to inefficient AD among the substrates tested. As raw SS was directly fermented to produce ethanol without AD, its net energy was the lowest in this study. This observation further demonstrated the contribution of efficient AD makes when co-producing methane and ethanol.

Table 3. Mass Balance of Energy Produced per Kg Dry Raw Feed

Feedstock	Energy Production (g)		Energy Input (kJ)		Energy Output (kJ)		Net Energy Balance (kJ)
	Methane [a]	Ethanol	AD [b]	EF [c]	AD [b]	EF [c]	
DMS (30 days)	91	98	-283	-1556	4550	2744	5455
PMS (30 days)	69.1	99	-253	-1572	3457	2772	4404
SS (30 days)	45.2	113.5	-147	-1802	2259	3178	3488
DMS (60 days)	115.3	88	-283	-1397	5765	2464	6549
SS (60 days)	80	108.9	-147	-1729	4003	3049	5176
SS (raw)	—	124	—	-1969	—	3472	1503

[a] Methane density is 0.77 g/L.
[b] Anaerobic digestion
[c] Ethanol fermentation

When considering the digestion time, digesting for 60 days achieved a higher net energy than digesting for 30 days, especially for SS. These results indicated that increasing the digestion time improved the net energy balance to an extent. Therefore, optimizing the parameters of this dual digestive process is important work that should be performed in the future to improve the efficiency of the co-production of methane and ethanol from lignocellulosic biomass.

CONCLUSIONS

1. Integrated methane and ethanol were produced from a mixture of soybean straw and livestock manures. The co-digestion of dairy manure and soybean straw (DMS) for 60 days resulted in optimal production.
2. Both feedstock and the digestion time played important roles in the integrated processing of methane and ethanol from livestock manure and soybean straw.
3. The results confirmed that the net energy captured from these integrated processes producing methane and ethanol was higher than that from processes that only produce ethanol.

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