Comparative Study of Chemical Pretreatments of Dairy Manure for Enhanced Biomethane Production

Qiang Yang, Hui Wang, Rebecca Larson, and Troy Runge*

Dairy manure containing partially digested plant cells is an inexpensive bioenergy feedstock. The carbohydrates and lignin that remain after digestion are typically processed in an anaerobic digester to produce biomethane, but due to the remaining material's recalcitrance, the process has a low conversion efficiency. To improve the conversion of this lignocellulosic material, chemical, thermal, or biological pretreatments can be considered. This study compared several chemical pretreatments including dilute acid, sulfite, and alkali pretreatments for dairy manure as a bioenergy feedstock and analyzed their impact on biomethane production. The comparative study showed that a hot alkali pretreatment (180 °C, 30 min) can improve the methane production of dairy manure by 50%, which is more effective than dilute acid (6.8%), sulfite (26.3%), and cold or ambient alkali (19.8 to 32.8%) pretreatments. However, the ambient alkali pretreatment (23 °C, 12 h) was calculated to be more economically feasible because of the net energy production.

Keywords: Dairy manure; Cellulose; Lignin; Hemicellulose; Pretreatment; Anaerobic digestion; Biomethane

*Contact information: Department of Biological Systems Engineering, University of Wisconsin-Madison, 460 Henry Mall, Madison, WI 53706, USA; *Corresponding author: trunge@wisc.edu*

INTRODUCTION

Animal manure is an abundant biomass feedstock, and thus is an inexpensive resource for bioenergy. Animal manure can be directly burned to produce heat and combustible syngas or pyrolyzed to bio-oil (Fernandez-Lopez *et al.* 2015). Animal manure contains partially digested plant cell walls and is comprised primarily of cellulose, hemicellulose, and lignin, which can be anaerobically digested to produce methane (Nasir *et al.* 2012; Babaee *et al.* 2013; Arikan *et al.* 2015; Sun *et al.* 2015). However, even after anaerobic digestion (AD), animal manure still has a significant amount of unconverted carbohydrates (Yang *et al.* 2016), which suggests that higher conversion is possible if the carbohydrates can be processed in the AD system.

During anaerobic digestion, manure carbohydrates are converted to organic acids, which are then biologically converted to methane and carbon dioxide. Cellulose, hemicellulose, and lignin are the major carbon sources in the feedstock for anaerobic microorganisms. However, lignin is not as biodegradable as cellulose and hemicellulose and is typically not converted during AD. Moreover, lignin can inhibit the access of microorganisms to cellulose and hemicellulose by forming a physical barrier (Kobayashi *et al.* 2004). Cattle manure is enriched in lignin content, as the carbohydrates have been largely consumed through digestion. As a result, the anaerobic digestion process of manure is typically slow, and the achievable methane yield is generally not satisfactory (Nasir *et al.* 2012, 2015).

An appropriate pretreatment can facilitate the anaerobic digestion of manure, and more importantly, can improve the methane yield (Nasir *et al.* 2015; Mancini *et al.* 2016). For example, biological pretreatments use aerobic fungi and bacteria to break down the recalcitrance of manure prior to anaerobic digestion. Physical pretreatments such as grinding and maceration can also facilitate anaerobic digestion and enhance the methane yield by increasing the available surface area of manure, which can improve the microbial access to the manure (Angelidaki and Ahring 2000). Microwave and ultrasonic-assisted thermal pretreatment also improve the anaerobic digestion performance of manure by breaking down the recalcitrant manure fiber (Jackowiak*et al.* 2011; Nilgun and Canan 2014). Unfortunately, the physical and thermal pretreatments of manure typically require high energy and thus may not provide net energy benefits. For example, Jiang *et al.* (2016) reported that the liquid hot water pretreatment obtained negative net electrical energy production from giant reed due to high energy input. In contrast, ambient alkali pretreated giant reed can achieve 27% higher net electrical energy production than that of untreated giant reed (Jiang *et al.* 2016).

Chemical pretreatment uses acid or alkaline additives to partially remove lignin and therefore increase the accessibility of carbohydrates to microbes (Salehian *et al.* 2013; Karray*et al.* 2015; Michalska *et al.* 2015; Papa *et al.* 2015; Jiang *et al.* 2016; Tsapekos *et al.* 2016). Combined physical and chemical pretreatment such as steam explosion also improves the methane yield of manure (Kobayashi *et al.* 2004; Bondesson *et al.* 2013). Comparative studies have shown that sulfite pretreatment is generally more effective than acid and alkaline pretreatments to improve enzymatic digestibility of lignocellulose. Such treatment works through sulfonation of the lignin to increase the hydrophilicity and allow extraction (Li *et al.* 2012; Yang and Pan 2012; Zhang *et al.* 2013). A previous study showed that sulfite pretreatment is more effective than the dilute acid and alkali pretreatments to improve the enzymatic saccharification of dairy manure (Yang *et al*. 2016).

For further information on pretreatments to enhance biogas production Zheng *et al*. (2014) provide a comprehensive review for lignocellulosic biomass and Ariunbaatar *et al.* (2014) for municipal solid waste. Although there has been considerable research related to pretreatment for anaerobic digestion, there have been no reported studies on the sulfite pretreatment for improving methane production. As this is a promising pretreatment for ethanol production, an investigation of this pretreatment on the methane production from dairy manure was warranted and was compared with other more well-known pretreatments. Additionally, because thermal pretreatments typically require high energy, and thus are less economically feasible, a cold alkali pretreatment was also investigated for improving the methane production from dairy manure.

EXPERIMENTAL

Materials

Undigested dairy manure samples (#1 and #2) were collected at a 4000 head dairy farm (Manitowoc County, WI, USA) and were air-dried prior to use. Two samples from this farm were acquired for these experiments to insure that fresh samples were used for each set of experiments. Fresh inoculum sludge samples after centrifugation (total solid (TS), 2.4%; total volatile solid, 1.6%; total organic carbon, 29.3% of TS; total nitrogen, 7.3% of TS; total phosphorus, 0.72% of TS; total potassium, 11.18% of TS; total sulfur, 0.29% of TS) were obtained from a different dairy that contained a digester (Dane County, WI, USA), and were used without additional nutrient addition. Sodium sulfite (SS), sulfuric acid (SA), and sodium hydroxide (NaOH, 50% w/w) were purchased from Thermo Fisher (Waltham, MA, USA). Acetic acid, propionic acid, butyric acid, thiourea (TU), and polyethylene glycol (PEG) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Pretreatment of Manure

The pretreatment conditions were reported previously (Yang *et al.* 2016) and are briefly summarized in Table 1. The hot chemical pretreatments were carried out in a 100 mL plastic vessel using a microwave reactor (Mars, CEM Corporation, Matthews, NC, USA). The cold chemical pretreatments were carried out in a 500 mL beaker. After pretreatments, the samples were washed with DI water and centrifugation until a neutral pH was achieved.

Anaerobic Digestion of Manure

Anaerobic digestion experiments were carried out in a 500 mL sterile glass bottle at 37 ± 0.1°C for 34 days on a continuously stirred (200 RPM), lab-scale anaerobic digester at a 5% (w/w) consistency (Jiang *et al.* 2016). Due to the varying treatments, which can change the N content, a constant inoculum/feed ratio of 0.64 was maintained, based on volatile solids. Batch experiments were carried out in duplicate to provide an estimate of standard error. Further replicates were not done due to equipment limitations. All experimental digestion samples were purged with nitrogen for 5 min to remove oxygen and sealed with butyl rubber stoppers. Biogas was measured through a Pulse-Flow Anaerobic/Anaerobic Respirometer (PF-8000, Respirometer Systems and Applications, Inc., Springdale, AR, USA). Biogas was collected in a Tedlar gas collection bag for composition analysis using a gas chromatograph. Volatile fatty acids in manure slurry after digestion were measured using a high-performance ion chromatograph.

The methane production potential, maximum methane production rate, and lag time were estimated according to the fit of the cumulative methane production data from the experiments to the modified Gompertz equation (Eq.1) (Lay *et al.* 1996),

$$B = B_o \times \exp\left\{-\exp\left[\frac{R_m \times e}{B_o}(\lambda - t) + 1\right]\right\} \tag{1}$$

where B is the cumulative methane yield at a given time (mL CH_4/gVS), B_o is the methane production potential (mL CH_4/gVS), R_m is the maximum methane production rate (mL CH_4/gVS/day), e is the mathematical constant (2.7183), λ is the lag time (days) for the methane production to begin, and t is the time (days).

Energy Calculation

The total energy production for the manure samples through combusting the produced biomethane was calculated using Eq. 2,

$$\textit{Total energy production} = B_o \times d \times HHV \tag{2}$$

where B_o is the measured methane production potential (L CH_4/kgTS), d is the methane density (0.000656 kg/L), and HHV is the higher heating value of methane (55 MJ/kg).

Respective energy consumptions for the manure collection and mixing and for the anaerobic digestion of manure were not considered in this study. Energy consumptions for the manure degradation reactions during pretreatments were also not considered. However, additional energy is needed for the manure pretreatment. Specifically, for the hot pretreatments, the manure samples (10 g, oven dried) were heated from 23 °C to 180 °C in 60 mL acidic or basic aqueous solutions; and for the ambient pretreatment, the manure sample (10 g, oven dried) was mixed with 50 mL alkali aqueous solution, and the resultant mixture was stored at 23 °C (Yang *et al*. 2016). The energy consumption for pretreating the manure sample was estimated using Eq. 3.

$$Energy\ consumption = (m_1 \Delta h + m_2 C_p \Delta T)/VS \quad (3)$$

where m_1 is the weight of water in the sample (0.060 kg), Δh is the change in specific enthalpy of water being heated from 23°C to 180°C (667 kJ/kg), m_2 is the weight of the manure (0.010 kg, oven dried), C_p is the specific heating capacity of manure (3.8 kJ/kg/°C) (Nayyeri *et al.* 2009), ΔT is the temperature increase from 23 °C to 180 °C, and VS the amount of volatile solids in the sample (0.008 kg). This simple estimation assumes recovery of required for latent heat of vaporization, while it neglects heat losses by the reactor and heats of reaction. Although limited in scope this estimate does provide a magnitude of the energy invested in the pretreatment to compare to the additional energy obtained. Full scale pretreatment systems would need to be designed and modeled to obtain a more accurate energy return on energy invested in pretreatments.

Analytic Methods

Total organic carbon (TOC), nitrogen (N), and sulfur (S) were analyzed using a LECO CNS-2000 Carbon, Nitrogen and Sulfur Analyzer (LECO Corporation, St. Joseph, MI, USA). Phosphorus (P) was analyzed using a UV-Vis spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) at 645 nm. Potassium (K) was measured using an ICP-OES spectrophotometer (SPECTRO Analytical Instruments, Inc., Kleve, Germany). Biogas composition was analyzed by a Gas Chromatograph SRI 8610C equipped with an FID detector (SRI Instruments, Inc., Torrance, CA, USA). Ash, extractives, and acid-insoluble (Klason) lignin were analyzed according to National Renewable Energy Laboratory Analytic Procedures (Sluiter *et al.* 2010). Total solids (TS) and volatile solids (VS) were analyzed according to published standards (APHA 2006). Monosaccharides (glucose, galactose, arabinose, mannose, and xylose) were measured by a high performance ion chromatography (HPIC, ICS-3000, Dionex, Sunnyvale, CA, USA) equipped with an integrated amperometric detector and Carbopac guard (PA20, 3 × 30 mm) and analytic (PA20, 3 × 150 mm) columns. Acetic acid, propionic acid, and butyric acid were measured using the Dionex ICS-3000 system equipped with a UV-vis detector and Supelcogel C-610H analytic (30 cm × 7.8 mm) and guard (5 cm × 4.6 mm) columns.

RESULTS AND DISCUSSION

Effect of Pretreatment on Composition and Nutrient

The composition of manure samples is shown in Table 2. The two manure samples had similar compositions. Extractives (materials that could be extracted from exhaustive ethanol Soxhlet extraction) for manure samples #1 and #2 were about 13% and 15%, respectively. Manure sample #2 contained higher volatile solids (86.0% *vs.* 81.8%) but lower total organic carbon (37.3% *vs.* 41.3%) than manure sample #1.

As summarized in Table 1, manure sample #1 was pretreated under hot conditions, and manure sample #2 was pretreated under cold conditions. Under hot pretreatment conditions, carbohydrates can be degraded into sugars, acetic acid, furfural, and hydroxymethylfurfural, while lignin can be degraded into aromatic compounds (Yang *et al*. 2016). The produced sugars can be anaerobically digested to produce methane. Although the formed furfural, hydroxymethylfurfural, and aromatic compounds can inhibit anaerobic digestion (Mousa and Forster 1999; Hernandez and Edyvean 2008; Pekařová*et al.* 2017), recycling spent pretreatment chemicals for reuse is economically important. Therefore, to exclude the effects of the produced furfural, hydroxymethylfurfural, and aromatic compounds and potentially recycle the spent chemicals for reuse, the pretreated manure samples in this study were washed to allow for direct comparison. The total solids, volatile solids, cellulose, hemicellulose, and lignin contents of the pretreated manure samples are reported in Table 2.

Table 1. Pretreatment Conditions of Manure

Pretreatment	Temperature (°C)	Time (h)	Chemicals
4% SA[a]	180	0.5	4% sulfuric acid
4% SA[a] + 9% SS[a]	180	0.5	9% sodium sulfite + 4% sulfuric acid
8% NaOH[a]	180	0.5	8% sodium hydroxide
7% NaOH[b] + 2% PEG[b]	23	12	7.0% sodium hydroxide + 2% polyethylene glycol
7% NaOH[b] + 5.5% TU[b]	-20	12	7.0% sodium hydroxide + 5.5% thiourea

[a]Chemical loading wt% based manure so oven-dry manure. [b]Chemical loading wt% of solution. SA-sulfuric acid; SS-sodium sulfite; NaOH-sodium hydroxide; PEG-polyethylene glycol; TU-thiourea.

Table 2. Yield and Composition of Untreated and Pretreated Manure Samples

Sample	Yield (%)	Gla (%)	Ara (%)	Xyl (%)	Glu (%)	KL (%)	TS (%)	VS (%)
untreated #1	N/A	1.8	2.2	18.2	23.2	23.1	93.5	81.8
4% SA	46.1	0.7	0.6	10.4	35.0	28.2	93.8	86.9
4% SA + 9% SS	48.3	1.6	2.3	20.5	37.1	22.3	81.7	72.4
8% NaOH	42.6	1.3	0.9	18.8	32.4	20.1	95.8	79.8
untreated #2	N/A	1.6	1.8	19.0	24.2	22.6	94.8	86.0
7% NaOH + 2% PEG	45.4	1.5	1.0	19.3	32.6	20.6	90.3	82.1
7% NaOH + 5.5% TU	46.2	1.6	1.2	20.2	31.8	21.9	88.1	84.4

Yield: Residual manure (oven-dry) as a weight percentage of initial manure (oven-dry). Gla-galactose; Ara-arabinose; Xyl-xylose; Glu-glucose; KL-Klason lignin; TS-total solid; VS-volatile solid;

Table 3. Effects of Pretreatment on Hemicellulose and Lignin in Manure Sample

Sample	Removal of Hemicellulose (%)	Removal of Lignin (%)
4% SA	75.7	43.7
4% SA + 9% SS	46.9	53.4
8% NaOH	59.7	62.9
7% NaOH + 2% PEG	55.8	58.6
7% NaOH + 5.5% TU	52.5	55.2

As expected, the chemical pretreatments noticeably changed the composition of the manure samples. In particular, the monomers associated with hemicellulose were reduced, suggesting hemicellulose degradation and removal during washing. As summarized in Table 3, the dilute acid pretreatment removed the most hemicellulose, followed by the alkali pretreatment and then the sulfite pretreatment. Therefore, the dilute acid pretreated manure sample has less hemicellulose than the original sample (12%), the sulfite (24%), hot alkali (21%), and cold alkali (22 to 23%) pretreated manure samples. Lignin was also greatly removed from the manure during the chemical pretreatments under both hot and cold conditions with the alkali pretreatments, removing more lignin than the acid pretreatments (Table 3). Therefore, the alkali pretreated manure exhibited lower (20 to 22%) lignin content than the acid pretreated sample (22 to 28%). Due to the partial removal of both lignin and hemicellulose, the manure sample was enriched in cellulose. Considering higher lignin and hemicellulose removal, the hot alkali pretreatment gave manure a relatively lower (43%) substrate yield than the dilute acid (46%), sulfite (48%), and cold alkali (45 to 46%) pretreatments. The pretreatments also changed the volatile solids of the manure samples, with a decrease observed for all chemical pretreatments except the dilute acid pretreatment. The decrease in volatile solids is primarily attributed to the removal of both lignin and hemicellulose that occurred from the pretreatment and washing. Interestingly, the dilute acid pretreatment increased the volatile solids of the manure sample from 82% to 87%. The pretreatments also changed the total organic carbon of the manure samples. The acidic (dilute acid and sulfite) pretreatments increased the total organic carbon, while the basic pretreatments decreased the total organic carbon.

Manure usually contains nutrients such as nitrogen, phosphorus, potassium, and sulfur, which were also characterized along with the Total Organic Carbon (TOC). As summarized in Table 4, nitrogen and potassium were partially removed from the manure samples during the pretreatments and washing, with the greatest loss in soluble potassium. Nitrogen and potassium are essential nutrients for the growth of anaerobic bacteria, but they can be inhibitory or toxic at high concentrations (Wang *et al.* 2014; Wu *et al.* 2016). The loss of nitrogen and potassium may influence the biomethane production. However, possibly due to accumulation, the inoculum sludge samples in this study contained relatively high contents of nitrogen and potassium, which likely provided sufficient nutrients for the anaerobic digestion of pretreated samples (Steinmetz *et al.* 2016; Wu *et al.* 2016). The loss of nitrogen and potassium during biomethane production in pretreated manure samples require further study.

Table 4. Nutrients of Manure Sample

Sample	TOC (%)	Total N(%)	NH_4-N(%)	Total P(%)	Total K(%)	Sulfur(%)
untreated #1	41.3	1.96	0.10	0.40	1.52	0.31
4% SA	44.3	1.97	0.10	0.38	0.06	0.26
4% SA + 9% SS	42.4	1.61	0	0.49	0.04	0.34
8% NaOH	39.6	0.80	0	0.51	0.07	0.24
untreated #2	37.3	1.50	0.20	0.52	0.82	0.23
7% NaOH + 2% PEG	35.7	0.60	0.30	0.38	0.09	0.19
7% NaOH + 5.5% TU	34.7	0.60	0.10	0.40	0.11	0.22

Effect of Pretreatment on Biomethane Production

Cumulative and daily methane productions of the manure (#1) samples before and after the dilute acid, sulfite, and hot alkali pretreatments are presented in Fig. 1. Their estimated methane production potentials, maximum production rates, and lag times are summarized in Table 5. The cumulative methane production was characterized by a sigmoid shaped curve, having a slow initial methane production with a significant increase, followed by a decline and eventual halt. As shown in Fig. 1 and Table 5, the time lag (the minimum time to produce methane, λ was the greatest at approximately 4 days for the untreated manure sample. In contrast, after the chemical pretreatments, the time lag was significantly reduced, indicating that the pretreated dairy manure is more readily digestible.

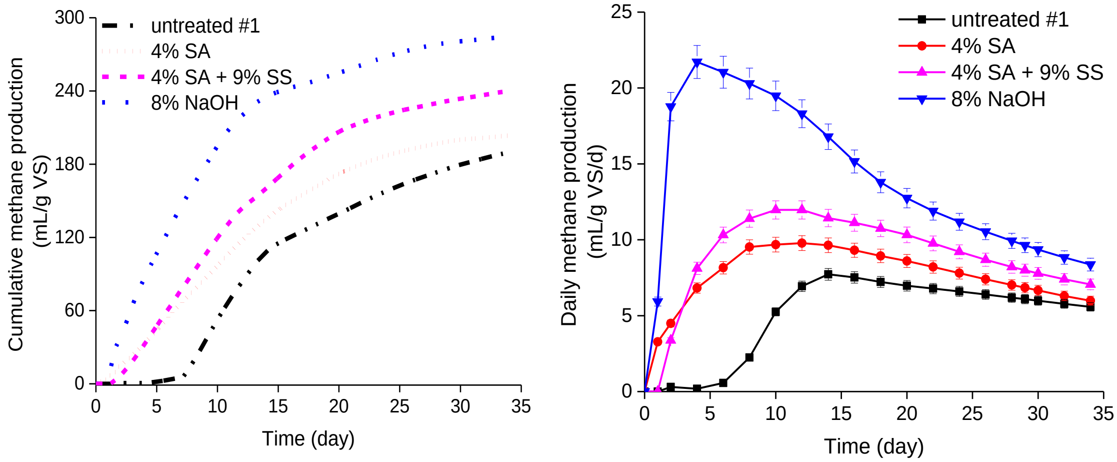

Fig. 1. Cumulative and daily methane production curves for untreated and pretreated dairy manure samples by dilute acid, sulfite, and hot alkali

Table 5. Estimated Methane Production Potential, Maximum Production Rate and Lag Time

Sample	B_0 (mL CH_4.g VS^{-1})		λ (d)		R_m (mL CH_4.g VS^{-1}. d^{-1})		R^2
	predicted	measured	predicted	measured	predicted	measured	
untreated #1	181 (0.3)	190 (0.9)	6.0	4.2	12.4	7.7 (0.8)	0.99
4% SA	206 (0.1)	203 (1.2)	1.8	0	11.6	9.8 (0.9)	0.99
4% SA + 9% SS	239 (0.2)	240 (1.2)	2.0	1	14.4	12.0 (0.9)	0.99
8% NaOH	277 (0.2)	285 (1.4)	1.0	0	19.9	22.0 (1.2)	0.99
untreated #2	212 (0.5)	192 (0.6)	0	0	8.2	12.0 (1.2)	0.99
7% NaOH + 2% PEG	281 (0.3)	255 (0.9)	0	0	10.8	15.3 (2.2)	0.99
7% NaOH + 5.5% TU	253 (0.9)	230 (0.8)	0	0	10.0	14.2 (1.5)	0.99

B_0: Methane production potential (mL CH_4/gVS) from biomethane production test. λ: Lag time (days) for methane production to begin. R_m: Maximum methane production rate (mL CH_4/gVS/day). All parameters are estimated according to the modified Gompertz equation through nonlinear regression using JMP Pro 11. Parenthesis are standard derivations.

Interestingly, all three of three of the 2nd set of experiments (untreated #2, and the NaOH experiments) predicted higher gas yields than measured. This was presumably due to the lack of lag time and initial high gas production of these samples, possibly due to the nature of inoculum use. However as both control manure samples produced approximately 190 mL of methane per gram of volatile solid (mL/g VS) after 35 days of digestion, comparison between studies can still be made, though with caution as the two sets of experiments show different biomethane production curves.

The chemically pretreated manure samples produced more methane mL/g VS over the same time periods, and thus exhibited greater digestibility and energy conversion. Particularly, the hot alkali pretreated manure sample produced approximately 285 mL/g VS methane, and the dilute acid and sulfite pretreated manure samples produced about 203 mL/g VS and 240 mL/g VS methane, respectively. Thus, the hot alkali, dilute acid, and sulfite pretreatments improved the methane production by 50%, 6.8%, and 26.3%, respectively. The hot alkali pretreated manure sample had the least lignin content (about 20%), which is likely the primary reason that sample produced the most methane. Similarly, because of the reduced lignin and enriched carbohydrates, the sulfite pretreated manure sample produced more methane than the dilute acid pretreated one. Additionally, the methane production rates of manure samples were much faster after the chemical pretreatments. For example, during the 35 days of digestion, the observed maximum methane production rates for the untreated, dilute acid, sulfite, and alkali pretreated manure samples were 7.7, 9.8, 12.0, and 22.0 mL CH_4/gVS/d, respectively.

Because the chemical pretreatments operate at high temperatures, especially the hot alkali, which can improve the accumulative methane production of manure up to 50%, they require high energy input and therefore may not be economically feasible. Thus, a pretreatment carried out at ambient or even a low temperature is preferred to improve the methane production of manure. Cold alkali pretreatments (*e.g.*, 7% sodium hydroxide aqueous solution containing 5.5% thiourea or 2% polyethylene glycol) greatly improve the enzymatic digestibility of manure as the treatment can partially remove lignin and reduce the crystallinity of cellulose via disrupting its hydrogen bond (Yang *et al*. 2016). Cold alkali pretreatments can have a similar effect though usually to a lesser degree. A 119% increase in the methane yield was observed for pine after a cold alkali pretreatment at 0 °C (Salehian *et al.* 2013). Mohsenzadeh *et al*. (2012) also reported that the methane yields of spruce and birch were improved by 56% and 600%, respectively, after a cold alkali pretreatment. Therefore, the manure samples in this study were pretreated by 7% alkali aqueous solution containing 5.5% thiourea (or 2% polyethylene glycol) at -20 °C (or room temperature, 23 °C) for 12 h.

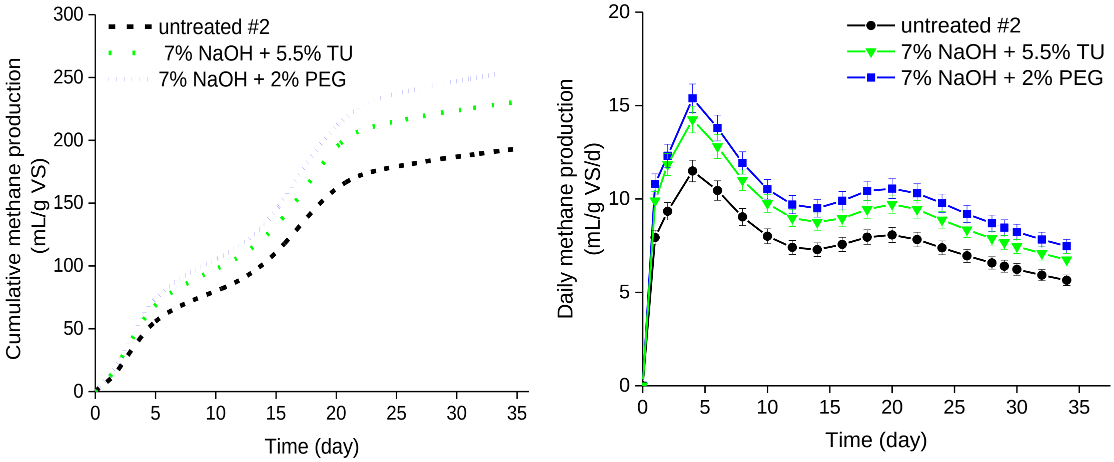

Fig. 2. Cumulative and daily methane production curves for untreated and pretreated dairy manure samples by cold alkali

Cumulative and daily methane production curves for untreated and pretreated manure samples by cold alkali are compared and presented in Fig. 2. The results show that the cold alkali pretreatment can greatly improve the methane production of manure. For example, after treatment with 7% alkali aqueous solution with 2% polyethylene glycol at 23 °C for 12 h, the methane production of the manure sample increased from about 192 to 255 mL/g VS. The approximately 33% improvement in the methane production is likely attributable to the lignin removal (about 59%) and possibly the disruption of crystallinity of cellulose (Salehian *et al.* 2013). The manure sample pretreated with 7% sodium hydroxide containing 5.5% thiourea at -20 °C showed contained lignin and less cellulose (Table 2), compared with the manure sample pretreated by the alkali aqueous solution with polyethylene glycol at 23 °C for 12 h. As a result, the alkali pretreatment with 7% sodium hydroxide containing 5.5% thiourea at -20 °C improved the methane production of manure by 19%, which is lower than that (33%) by the alkali pretreatment with 7% sodium hydroxide containing 2% polyethylene glycol at 23 °C for 12 h.

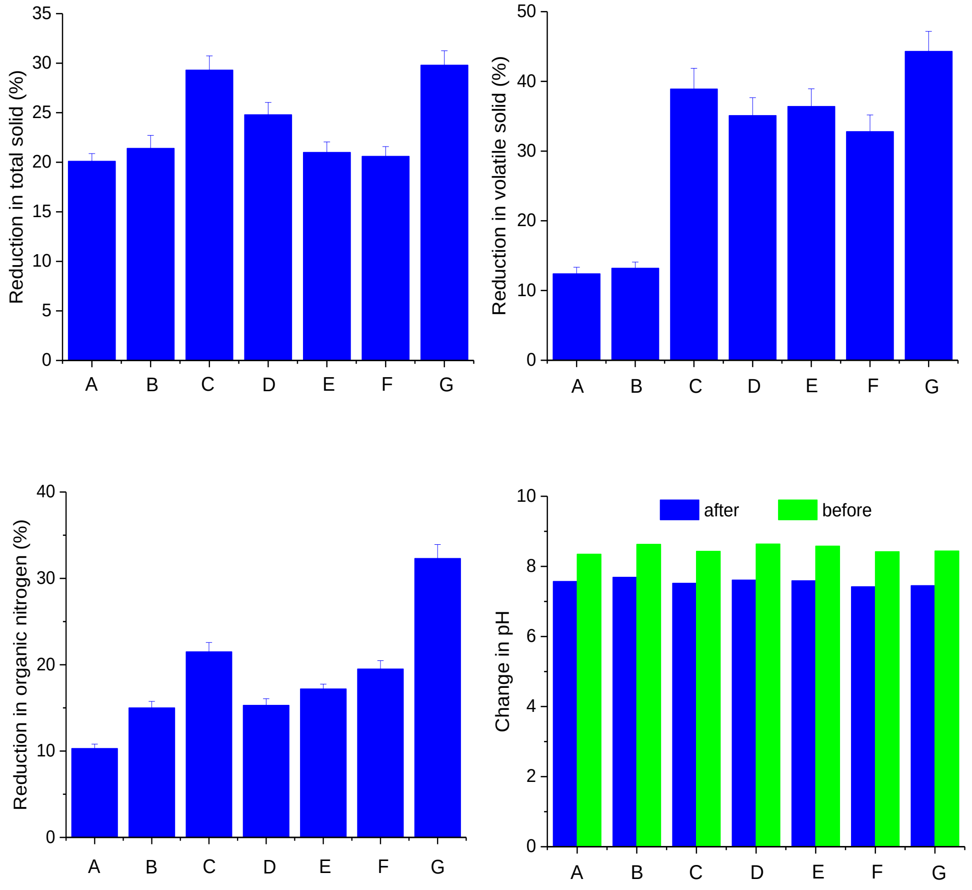

Fig. 3. Changes in total solids, volatile solids, organic nitrogen, and pH value after the 35-day anaerobic digestion period. A-untreated #1; B-untreated #2; C-8% NaOH; D-7% NaOH + 2% PEG; E-7% NaOH + 5.5% thiourea; F-4% SA; G-4% SA + 9% SS.

After anaerobic digestion, the total and volatile solids of the manure samples were greatly reduced, as shown in Fig. 3, with the pretreated manure samples generally having a greater reduction in the total and volatile solids than the untreated manure samples. During anaerobic digestion, the nitrogen in amino acids can be converted to ammonia and lost as a gas. Phosphorus and potassium were not significantly changed (data not shown), as expected (Aguirre-Villegas *et al.* 2014). As the anaerobic digestion proceeds, the volatile solids are reduced to intermediate volatile fatty acids (VFAs). The initial pH value deceases because of the accumulation of VFAs and then increases after conversion of VFAs to methane. In this study, the initial pH value of the manure sample solution after digested for 35 days generally decreased from 8.5 to 7.5, as shown in Fig. 3.

To estimate whether the chemical pretreatments used in this study are economically feasible, the respective energy productions and consumption were simply estimated, and the results are summarized in Table 6. The hot pretreated manure samples generated less net energy than the untreated controls, while the ambient pretreated manure sample achieved 33.1% higher net energy than that of the untreated manure sample.

Table 6. Estimated Net Energy Production for Untreated and Pretreated Manure Samples

Sample	Energy Production (kJ/kg TS)	Pretreatment Energy * (kJ/kg TS)	Net Energy (kJ/kg TS)
untreated #1	6052	0	6052
4% SA	6847	5002	1845
4% SA + 9% SS	7743	5002	2741
8% NaOH	8643	5002	3641
untreated #2	6341	0	6341
7% NaOH + 2% PEG	8440	0	8440

* The pretreatment energy input is estimated from a simple model of the heat energy required to heat the material from 23 °C to 180 °C, as described in the methodology section.

Based on the results from this study, which compared several chemical pretreatments including dilute acid, sulfite and alkali pretreatments for dairy manure, it appears that the alkali pretreatments are more effective than the dilute acid or sulfite. Additionally, the ambient temperature pretreatment seems to provide similar benefits as hot pretreatment, but is likely more feasible because of the lower energy inputs required.

CONCLUSIONS

1. A chemical pretreatment can greatly improve the methane production of dairy manure, primarily through partial removal of lignin.
2. The sulfite pretreatment is better than the dilute acid (with same amount of sulfuric acid) pretreatment, while the hot alkali pretreatment outperforms the cold alkali, dilute acid, and sulfite pretreatments.
3. The ambient alkali pretreatment was the most promising treatment when considering the net energy production of the system.

ACKNOWLEDGMENTS

The authors are grateful for the support of the United States Department of Agriculture-National Institute of Food and Agriculture (Grant number 2012-10006-19423) and the Wisconsin Energy Institute for their financial support.

REFERENCES CITED

Angelidaki, I, and Ahring, B. K. (2000). “Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure,” *Water Science and Technology* 41(3), 189-194.DOI: 10.4172/2252-5211.1000248

Aguirre-Villegas, H. A., Larson, R., and Reinemann, D. J. (2014). “From waste-to-worth: energy, emissions, and nutrient implications of manure processing pathways,” *Biofuels, Bioproducts and Biorefining* 8(6), 770-793. DOI: 10.1002/bbb

APHA (2006). “Standard methods for the examination of water and wastewater,” American Public Health Association, Washington, D.C.

Arikan, O. A., Mulbry, W., and Lansing, S. (2015). “Effect of temperature on methane production from field-scale anaerobic digesters treating dairy manure,” *Waste Management* 43, 108-113. DOI: 10.1016/j.wasman.2015.06.005

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., and Lens, P. N. L. (2014). "Pretreatment methods to enhance anaerobic digestion of organic solid waste," *Applied Energy* 123, 143-156. DOI: 10.1016/j.apenergy.2014.02.035

Babaee, A., Shayegan, J., and Roshani, A. (2013). “Anaerobic slurry co-digestion of poultry manure and straw: Effect of organic loading and temperature,” *Journal of Environmental Health Science & Engineering* 11(1), 1-6. DOI: 10.1186/2052-336X-11-15

Bondesson, P. M., Galbe, M., and Zacchi, G. (2013). “Ethanol and biogas production after steam pretreatment of corn stover with or without the addition of sulphuric acid,” *Biotechnology for Biofuels* 6:11. DOI: 10.1186/1754-6834-6-11

Fernandez-Lopez, M., Puig-Gamero, M., Lopez-Gonzalez, D., Avalos-Ramirez, A., Valverde, J., and Sanchez-Silva, L. (2015). “Life cycle assessment of swine and dairy manure: pyrolysis and combustion processes,” *Bioresource Technology* 182, 184-192. DOI: 10.1016/j.biortech.2015.01.140

Hernandez, J. E., and Edyvean, R. G. J. (2008). “Inhibition of biogas production and biodegradability by substituted phenolic compounds in anaerobic sludge,” *Journal of Hazardous Materials* 160 (1), 20-28. DOI: 10.1016/j.jhazmat.2008.02.075

Jackowiak, D., Bassard, D., Pauss, A., and Ribeiro, T. (2011). “Optimization of a microwave pretreatment of wheat straw for methane production,” *Bioresource Technology* 102(12), 6750-6756. DOI: 10.1016/j.biortech.2011.03.107

Jiang, D. P., Ge, X. M., Zhang, Q. G., and Li, Y. B. (2016). “Comparison of liquid hot water and alkaline pretreatments of giant reed for improved enzymatic digestibility and biogas energy production,” *Bioresource Technology* 216, 60-68. DOI: 10.1016/j.biortech.2016.05.052

Karray, R., Hamza, M., and Sayadi, S. (2015). “Evaluation of ultrasonic, acid, thermo-alkaline and enzymatic pre-treatments on anaerobic digestion of *Ulva rigida* for biogas production,” *Bioresource Technology* 187, 205-213. DOI: 10.1016/j.biortech.2015.03.108

Kobayashi, F., Take, H., Asada, C., and Nakamura, Y. (2004). “Methane production from steam-exploded bamboo,” *Journal of Bioscience and Bioengineering* 97(6), 426-428. DOI: 10.1016/S1389-1723(04)70231-5

Lay, J. J., Li, Y. Y., and Noike, T. (1996). “Effect of moisture content and chemical nature on methane fermentation characteristics of municipal solid wastes,” *Journal of Environmental System Engineering JSCE*552 (VII), 101-108.

Li, Z. Q., Yang, Q., Jiang, Z. H., Fei, B. H., Cai, Z. Y., and Pan, X. J. (2012).

"Comparative study of sulfite (SPORL), dilute acid and NaOH pretreatments of bamboo for enzyamtic saccharification," *Journal of Biobased Materials and Bioenergy* 6(5), 544-551.DOI: 10.1166/jbmb.2012.1250

Mancini, G., Papirio, S., Lens, P. N. L., and Esposito, G. (2016). "Solvent pretreatments of lignocellulosic materials to enhance biogas production: a review," *Energy Fuels* 30(3), 1892-1903. DOI:10.1021/acs.energyfuels.5b02711

Michalska, K., Bizukojć, M., and Ledakowica, S. (2015). "Pretreatment of energy crops with sodium hydroxide and cellulolytic enzymes to increase biogas production," *Biomass and Bioenergy* 80, 213-221.DOI:10.1016/j.biombioe.2015.05.022

Mohsenzadeh, A., Jeihanipour, A., Karimi, K., and Taherzadeh, M. J. (2012). "Alkali pretreatment of softwood spruce and hardwood birch by NaOH/thiourea, NaOH/urea, NaOH/urea/thiourea, and NaOH/PEG to improve ethanol and biogas production," *Journal of Chemical Technology and Biotechnology* 87(8), 1209-1214. DOI: 10.1002/jctb.3695

Mousa, L., and Forster, C. F. (1999). "The use of glucose as a growth factor to counteract inhibition in anaerobic digestion," *Process Safety and Environmental Protection* 77(B4), 193-198. DOI: 10.1205/095758299530062

Nasir, I.M., Mohd Ghazi, T.I., and Omar, R. (2012). "Anaerobic digestion technology in livestock manure treatment for biogas production," *Engineering in Life Sciences* 12(3), 258-269. DOI: 10.1002/elsc.201100150

Nasir, I. M., and Mohd Ghazi, T.I. (2015). "Pretreatment of lignocellulosic biomass from animal manure as a means of enhancing biogas production," *Engineering in Life Sciences* 15(7), 733-742. DOI:10.1002/elsc.201500019

Nayyeri, M. A., Kianmehr, M. H., Arabhosseini, A., and Hassan-Beygi, S. R. (2009). "Thermal properties of dairy cattle manure," *International Agrophysics* 23(4), 359-366.

Nilgun, A. O., and Canan, C. Y. (2014). "Ultrasound assisted biogas production from landfill leachate," *Waste Management* 34(7), 1165-1170.DOI: 10.1016/j.wasman.2014.03.003

Papa, G., Rodriguez, S., George, A., Schievano, A., Orzi, V., Sale, K. L., Singh, S., Adani, F., and Simmons, B. A. (2015). "Comparison of different pretreatments for the production of bioethanol and biomethane from corn stover and switchgrass," *Bioresource Technology* 183, 101-110. DOI: 10.1016/j.biortech.2015.01.121

Pekařová, S., Dvořáčková, M., Stloukal, P., Ingr, M., Šerá, J., and Koutny, M. (2017). "Quantitation of the inhibition effect of model compounds representing plant biomass degradation products on methane production," *BioResources* 12(2), 2421-2432. DOI: 10.15376/biores.12.2.2421-2432

Salehian, P., Karimi, K., Zilouei, H., and Jeihanipour, A. (2013). "Improvement of biogas production from pine wood by alkali pretreatment," *Fuel* 106, 484-489. DOI: 10.1016/j.fuel.2012.12.092

Sluiter, J. B., Ruiz, R. O., Scarlata, C. J., Sluiter, A. D., and Templeton, D. W. (2010). "Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods," *Journal of Agricultural and Food Chemistry* 58 (16), 9043-9053. DOI:10.1021/jf1008023

Sun, C., Cao, W. X., and Liu, R. H. (2015). "Kinetics of methane production from swine manure and buffalo manure," *Applied Biochemistry and Biotechnology* 177(4), 985-995. DOI: 10.1007/s12010-015-1792-y

Steinmetz, R. L. R., Mezzari, M. P., da Silva, M. L. B., Kunz, A., do Amaral, A. C., Tápparo, D. C., and Soares, H. M. (2016). "Enrichment and acclimation of an anaerobic mesophilic microorganism's inoculum for standardization of BMP assays," *Bioresource Technology* 219, 21-28. DOI: 10.1016/j.biortech.2016.07.031

Tsapekos, P., Kougias, P. G., Frison, A., Raga, R., and Angelidaki, I. (2016). "Improving methane production from digested manure biofibers by mechanical and thermal alkaline pretreatment," *Bioresource Technology* 216, 545-552. DOI: 10.1016/j.biortech.2016.05.117

Wang, X. J., Lu, X. G., Li, F., and Yang, G. H. (2014). "Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition," *PLOS ONE*9(5), e97265, 1-7. DOI: 10.1371/journal.pone.0097265

Wu, L. J., Kobayashi, T., Kuramochi, H., Li, Y. Y., and Xu, K. Q. (2016). "Effects of potassium, magnesium, zinc, and manganese addition on the anaerobic digestion of de-oiled grease trap waste," *Arabian Journal for Science and Engineering* 41(7), 2417-2427. DOI 10.1007/s13369-015-1879-3

Yang, Q., and Pan, X.J. (2012). "Pretreatment of *Agave americana* stalk for enzymatic saccharification," *Bioresource Technology* 126, 336-340. DOI:10.1016/j.biortech.2012.10.018

Yang, Q., Zhou, S. F., and Runge, T. (2016). "Dairy manure as a potential feedstock for cost-effective cellulosic bioethanol," *BioResources* 11(1), 1240-1254. DOI: 10.1002/elsc.201100150

Zhang, D. S., Yang, Q., Zhu, J. Y., and Pan, X. J. (2013). "Sulfite (SPORL) pretreatment of switchgrass for enzymatic saccharification," *Bioresource Technology* 129, 127-134. DOI: 10.1016/j.biortech.2012.11.031

Zheng, Yi, Zhao, J., Xu, F. Q., and Li, Y. B. (2014). "Pretreatment of lignocellulosic biomass for enhanced biogas production," *Progress in Energy and Combustion Science* 42, 35-53. DOI: 10.1016/j.pecs.2014.01.001

Article submitted: May 20, 2017; Peer review completed: June 30, 2017; Revised version received and accepted: August 11, 2017; Published: August 22, 2017.
DOI: 10.15376/biores.12.4.7363-7375