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Anaerobic co-digestion of forage radish and dairy manure in complete mix digesters

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HIGHLIGHTS

- Co-digestion of manure and forage radish was tested in mixed digesters (850 L).
- Methane production increased by 39% with radish addition compared to manure only.
- Radish co-digestion lowered the H₂S concentration of the biogas.
- Extrapolated to a 200-cow dairy, co-digestion could produce 3150 m³ CH₄/month.

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ABSTRACT

Pilot-scale digesters (850 L) were used to quantify CH₄ and H₂S production when using forage radish cover crops as a co-digestion feedstock in dairy manure-based digesters. During two trials, triplicate mixed digesters were operated in batch mode with manure-only or radish + manure (27% and 13% radish by wet weight in Trial 1 and 2, respectively). Co-digestion increased CH₄ production by 11% and 39% in Trial 1 and 2, respectively. As H₂S production rapidly declined in the radish + manure digesters, CH₄ production increased reaching high levels of CH₄ (≥67%) in the biogas. Over time, radish co-digestion lowered the H₂S concentration in the biogas (0.20%) beyond that of manure-only digestion (0.34–0.40%), although cumulative H₂S production in the radish + manure digesters was higher than manure-only. Extrapolated to a farm-scale (200 cows) continuous mixed digester, co-digesting with radish could generate 3150 m³ CH₄/month, providing a farmer additional revenue up to \$3125/month in electricity sales.

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1. Introduction

Dairy manure has a low energy density in comparison to other anaerobic digestion (AD) feedstocks and economic returns from manure-only digestion are often low to negative (Klavon et al., 2013; Wang et al., 2011). However, biogas production from dairy digesters can become a more economically viable practice by using additional biodegradable feedstocks located in close proximity to the dairy facility (El-Mashad and Zhang, 2010). Co-digesting dairy manure with other substrates, such as fats, oils, and grease (Lansing et al., 2010), slaughterhouse waste (Alvarez and Liden, 2008), or energy crops (Amon et al., 2007) with higher biogas potential have been shown to increase biogas production by 100–500% (El-Mashad and Zhang, 2007; Lansing et al., 2010), thus increasing the feasibility of AD technology, especially for small to

mid-sized dairy farmers (Klavon et al., 2013). Energy crop digestion is increasingly utilized due to the higher methane (CH₄) yield relative to animal manure (120–300 L CH₄/kg VS) (Al Seadi et al., 2008; Lansing et al., 2010). Some of the most widely used energy crops are maize (205–450 L CH₄/kg VS) (Bruni et al., 2010; Braun et al., 2009), switchgrass (298–467 L CH₄/kg VS) (Masse et al., 2010; Braun et al., 2009), sugar beets (236–381 L CH₄/kg VS) (Umetsu et al., 2006; Braun et al., 2009), sunflower grass (154–400 L CH₄/kg VS), and Sudan grass (213–303 L CH₄/kg VS) (Amon et al., 2007; Braun et al., 2009).

Forage radish is listed as a top cover crop species for the Northeast and Mid-Atlantic regions of the United States (SARE, 2007), with over 15,000 acres planted in Winter 2012 in the state of Maryland alone. Traditionally, corn-silage based dairy farmers leave the land fallow after harvesting corn silage in August. Planting forage radish as a winter cover crop immediately after corn harvest will not interfere with food supply. After several consecutive nights with temperatures below –6 °C, the radish cover

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crop winter-kills and rapidly decays, leaving behind a clean and enhanced seedbed. Utilizing radish as a winter cover crop results in multiple soil and environmental benefits, such as erosion control, improved soil fertility, and the alleviation of soil compaction (Gruver et al., 2014).

Forage radish is a Brassica crop and has a relatively high sulfur content (7.8–8.2 g/kg dry matter in the shoots) (Lounsbury, 2013). During AD, organic sulfur decomposition leads to hydrogen sulfide (H₂S) production, as sulfate reducing bacteria (SRB) utilize acetate to reduce SO₄²⁻ to H₂S. The most common pathway for CH₄ production is the acetoclastic pathway, where methanogens split acetate to form CH₄ and CO₂. Since SRB and methanogens both utilize acetate and H₂ as primary substrates, competition for available resources can occur during AD (Liu and Whitman, 2008). As sulfate reduction is more energetically favorable (–152 kJ/mol) than methanogenesis (CO₂ reduction: –131 kJ/mol; acetoclastic pathway: –37 kJ/mol) (Madigan et al., 2012), CH₄ production can be suppressed when H₂S production is high due to substrate limitation (Liu and Whitman, 2008). For example, Khanal and Huang (2003) found that during anaerobic treatment of high-sulfate wastewater, increasing sulfate concentration from 1000 to 5000 mg/L significantly increased H₂S production and decreased CH₄ production by 50%.

Hydrogen sulfide reacts with water vapor present in the biogas producing sulfuric acid, which can corrode piping and engine units. The H₂S content of biogas generated from animal manure ranges from 1000 to 3000 ppm (0.10–0.30%) (Al Seadi et al., 2008). The end use of the biogas dictates the extent to which the H₂S must be removed prior to usage. In combined heat and power (CHP) engines, the H₂S concentration should not exceed 100–500 ppm (0.01–0.05%) to prevent corrosion (Deublein and Steinhauser, 2011). Considering that biogas engines are often the largest cost associated with AD systems (Klavon et al., 2013) and that the majority of AD systems in the U.S. use the produced biogas for CHP or sole electricity production (AgSTAR, 2013), it is imperative that the H₂S concentration is not drastically increased when digesting sulfur-rich feedstocks, such as forage radish.

A major barrier to AD installation in the U.S. has been the lack of data on biogas potential in the literature, especially at the field-scale level. The data that is available in the literature focuses primarily on CH₄ production, with less attention to the effect of feedstock selection on H₂S production. This research investigates forage radish cover crops as a renewable source of energy in terms of CH₄ production, the effect of radish co-digestion on H₂S production, and the relationship between H₂S production and methanogenesis limitations. Specifically, this research seeks to determine if additional benefits can be obtained from the forage radish by harvesting the above-ground material prior to winter kill and utilizing it as a co-substrate in dairy digesters to increase CH₄ production. The overall objectives of this research were: (1) to determine potential CH₄ and H₂S production when co-digesting forage radish cover crops with dairy manure in batch pilot-scale complete mix digesters, (2) to determine how the percentage of forage radish in the co-digestion mixture affects CH₄ production, and (3) to quantify the radish crop acreage required for co-digestion at the farm-scale level and how inclusion of radish cover crops affects

on-farm energy production potential. The results can be used by dairy farmers to maximize CH₄ production in digesters during the winter when the demand for supplemental heating is the greatest.

2. Methods

Pilot-scale complete mix anaerobic digesters were designed and constructed to evaluate the anaerobic co-digestion of forage radish (*Raphanus sativus* var. *longipinnatus*) and the liquid fraction of solids-separated dairy manure under field conditions. The research was conducted at the USDA Beltsville Agricultural Research Center (BARC) dairy farm (39.03°, –76.89°) located in Beltsville, Maryland.

2.1. Feedstocks

Forage radish was grown as a cover crop immediately after corn silage harvest, sown in August and harvested in early December prior to a predicted hard freeze. To harvest, the cover crop was first mowed with a rotary mower as close to ground level as practical (3–5 cm). This mow cut the leafy shoots plus a portion of the fleshy root that extended above ground into a windrow. A forage chopper then passed over the windrow to harvest the above-ground biomass and the chopped material was blown into an adjacent wagon for collection. During this process, the radish biomass was transformed into a semi-slurry state. The harvested forage radish biomass was stored in sealed plastic buckets and frozen until use. Due to the heterogeneity of the harvested radish particle size (up to 10 cm), an industrial vertical cutter (Hobart Corporation; Troy, Ohio USA; model VCM-40) was used to further reduce the radish particle size to less than 3 cm to prevent damaging the pumping system and clogging the digester piping during digester loading.

Solids-separated dairy manure was obtained from BARC's dairy research unit. This 120-cow dairy uses a scrape system to collect raw manure and a FAN separator (0.64 cm mesh screen) to remove roughly 80% of the solids prior to treatment in a mesophilic complete mix digester (540 m³). The solids-separated dairy manure was collected from the holding pit on three different dates for the experiments. Total solids content of three sampling events varied 2–4%, primarily due to differences in water usage in the barn. Inoculum used in the batch digesters to accelerate biogas production was obtained from a sampling port located inside the BARC complete mix digester. The BARC digester is fed daily with the liquid fraction of solids-separated dairy manure and operates at 25–35 °C. The inoculum substrate from inside the BARC digester had an average pH of 7.5, and total solids (TS) and volatile solids (VS) concentration of 24 and 15 mg/g, respectively (Table 1).

2.2. Experimental design

Six pilot-scale complete mix anaerobic digesters were fabricated from 850 L (working volume) high-density polyethylene conical tanks (Ace Roto-Mold; Hospers, Iowa USA) equipped with silicone adhesive rubber heating blankets (BriskHeat; Columbus, Ohio USA; model SRP series) and radiant foil shells to maintain a

Table 1
Feedstock characterization represented by average (±standard error).

	M1		RM1 (27% radish:73% manure)			M2 and RM2 (13% radish:87% manure)		
	Inoculum	Manure	Inoculum	Manure	Radish	Inoculum	Manure	Radish
pH	7.5 (0.03)	6.9 (0.02)	7.5 (0.02)	6.9 (0)	4.6 (0.1)	7.5 (0.01)	7.2 (0.01)	4.3 (0.01)
sCOD (g/L)	3.95 (0.01)	11.9 (0.03)	4.08 (0.07)	12.8 (0.03)	51.9 (0.3)	3.15 (0.05)	8.03 (0.14)	51.2 (0.1)
TS (mg/g)	25.7 (0.1)	30.8 (0.4)	25.6 (0.02)	35.2 (0.02)	112 (4)	20.6 (0.1)	23.6 (0.1)	106 (0.4)
VS (mg/g)	16.0 (0.1)	22.3 (0.3)	16.0 (0.01)	26.1 (0.1)	89.6 (3.2)	12.7 (0.1)	17.2 (0.1)	81.4 (0.3)

Table 2
Feedstock loading.

	M1	RM1 (27% radish:73% manure)	M2	RM2 (13% radish:87% manure)
Inoculum (kg _{ww})	411	411	411	411
Manure (kg _{ww})	365	268	365	317
Radish (kg _{ww})	0	97	0	49
Total (kg _{ww})	776	776	776	776
Inoculum (kg VS)	6.58	6.58	5.34	5.34
Manure (kg VS)	8.04	6.98	6.21	5.39
Radish (kg VS)	0	8.74	0	3.98
Total (kg VS)	14.6	22.3	11.6	14.7
ISR ^a	1:1.2	1:2.4	1:1.2	1:1.8

^a Inoculum to substrate ratio (ISR) calculated on a VS basis.

35°C digestion temperature. Custom made top-mounted stirrers (using 1/15 hp Dayton right angle gear motors driving 25 cm diameter beveled mixing blades at 22 rpm) were used to agitate the contents twice daily for 15-min periods. Two field trials were conducted using six digesters operating in batch mode for 33 days, which corresponded to the time period in which large decreases in daily biogas production were observed, with <1% of the cumulative biogas production being produced daily. After the first 33-day trial, the six digesters were emptied and cleaned before being refilled for the second trial. For each trial, all digesters were loaded on the same day with the exception of the manure-only controls in Trial 1, which were loaded the following day. The digesters were operated in batch mode. All digesters contained 776 kg of total feedstock (Table 2). The dairy's truck scale (± 20 lbs) was used to weigh all feedstocks into a secondary container. Previous experiments demonstrated that the radish had a tendency to settle out of the manure. Therefore, the feedstocks were manually stirred in the secondary container while being transferred with a 1.5 hp centrifugal pump (Dayton; China) into each digester.

Results from previous laboratory-scale co-digestion experiments using dairy manure and forage radish mixtures showed that the optimal inoculum to substrate ratio (ISR) for radish and manure digestion was 65:35 on a wet weight (ww) basis (data unpublished). Due to differences in the VS content of the feedstocks during the laboratory and field trials, the ISR was adjusted for the pilot-scale experiment. All complete mix digesters were loaded in each field trial with 53% inoculum and 47% substrate (ww basis), with the substrate consisting of manure-only (control) or radish + manure (Table 2). Since the VS content of forage radish is over three times greater than dairy manure, the total amount of substrate (ww basis) added to each digester was kept constant to avoid substrate quantity being a confounding variable in the comparative study. As a result, the digesters had different VS contents. To make comparisons between treatments, the biogas data was normalized by the amount of VS added.

For Trials 1 and 2, three digesters contained manure-only (M1 and M2, respectively) and had an ISR of 1:1.2 (VS basis). For Trial 1, three radish + manure digesters contained 27% radish and 73% dairy manure (ww) (RM1), which corresponded to an ISR of 1:2.4. For Trial 2, the three radish + manure digesters (RM2) contained 13% radish and 87% dairy manure (ww), which corresponded to an ISR of 1:1.8 (Table 2).

2.3. Biogas analysis

The biogas generated was quantified with gas flow meters (EKM Metering Inc.; Santa Cruz, California USA; model EKM-PGM.75). Biogas samples were taken at least weekly from each digester with a syringe, placed into pre-evacuated foil gas bags, and analyzed for CH₄ and H₂S content using a gas chromatograph (Agilent Technol-

ogies, Inc.; Shanghai China; model 7890 A) with a thermal conductivity detector at 250 °C with an HP-Plot Q capillary column (Agilent J&W; USA) and He as the carrier gas at 8.6 ml/min. The oven operated at 60 °C for 2 min and subsequently ramped at 30 °C/min to 240 °C.

2.4. Feedstock characterization

The digester feedstocks were characterized prior to digestion (Table 1). Liquid digester samples were collected weekly and before and after each trial. Samples were analyzed for pH, soluble chemical oxygen demand (sCOD), total and volatile solids (TS, VS), total Kjeldahl nitrogen (TKN), total Kjeldahl phosphorus (TKP), and total sulfur. The pH was determined with an Accumet AB 15 pH meter. Standard Methods for the Examination of Water and Wastewater (APHA, 2005) were used to determine TS (Method 2540B) and VS (Method 2540E). The reactor COD digestion method was adapted by HACH Method 8000 for sCOD with 1.5 µm filtrate used for the analysis. A Lachat (QuickChem 8500 Series 2 FIA Automated Ion Analyzer) was used to determine TKN and TKP after Kjeldahl digestion with concentrated H₂SO₄ and CuSO₄*5H₂O and filtration through 0.45 µm membranes (QuikChem Method 13-107-06-2-D for TKN and 13-115-01-1-B for TKP). Composite samples from each digester type (manure-only or radish + manure) were submitted to Cumberland Valley Analytical Services (Hagerstown, MD) for total sulfur analysis and analyzed according to Standard Methods.

2.5. Statistical analysis

The experimental design for the two independent trials was a complete randomized design with six experimental units (digesters) and two treatment levels (presence or absence of radish) with three replicates each. A single factor ANOVA followed by Tukey–Kramer's post hoc test showed a significant difference between the CH₄ yields in the manure-only digesters (controls) in Trials 1 and 2. Therefore, statistical comparisons were only made within each trial. Significant differences within each trial were determined with *t*-tests for average CH₄ yields, H₂S yields, sCOD, TKN, and TKP using SAS 9.3 (SAS, Cary, NC) with an alpha of 0.05. Reported values are given as means with standard errors.

3. Results and discussion

3.1. CH₄ production

Co-digestion of forage radish cover crops in dairy manure complete mix batch digesters increased CH₄ production relative to that from digesters containing only dairy manure. The average CH₄ production value for RM1 (12.81 L CH₄/kg substrate) was 68% greater than M1 (7.61 L CH₄/kg substrate) (*p*-value = 0.003) and RM2

(8.38 L CH₄/kg substrate) was 77% greater than M2 (4.74 L CH₄/kg substrate) (p -value = 0.001) when normalized by kilograms of substrate added (Fig. 1; Table 3). RM2 (13% radish) contained half the radish content of RM1 (27% radish), thus having a reduced VS load in comparison to RM1.

The radish + manure digesters initially experienced a lag in CH₄ production compared to the manure-only digesters. Normalized by VS addition, RM1 and RM2 required approximately 15 and 7 days, respectively, to equal the CH₄ production of M1 and M2, respectively (Fig. 1). Additionally, in Days 1–3, RM1 and RM2 had <50% CH₄ in the biogas. However, by the time the digesters reached peak CH₄ production, the CH₄ concentration of RM1 and RM2 had increased to 76% and 67%, respectively. Overall, the CH₄ concentration of the biogas for RM1 and RM2 was significantly higher than the respective manure-only digesters: M1 and M2 (p -value = 0.012 and 0.045, respectively).

Although the dairy manure obtained for Trial 2 was more dilute due to increased use of misting units in the dairy barn, we expected that the CH₄ production values for M1 and M2 would be similar when normalized by VS added considering that each contained the same quantity of inoculum and manure (Fig. 1; Table 2). In contrast to our expectations, average CH₄ production values from M1 (190 L CH₄/kg VS) and M2 (150 L CH₄/kg VS) were significantly different (p -value = 0.002). For this reason, differences in CH₄ production between the two trials could not be attributed only to changes in radish content and statistical comparisons could only be conducted within each trial. Although we cannot explain

the basis for the differences in CH₄ production between M1 and M2, the more dilute manure in Trial 2 could have resulted in substrate limitations, or other factors such as nutrient availability or toxicity could have affected the digestion process.

When normalizing CH₄ production by VS, the CH₄ production values for M1 and RM1 differed by only 11% and were not statistically different (190 and 210 L CH₄/kg VS, respectively; p -value = 0.21). In Trial 2, there was a statistically significant difference between M2 and RM2, with a 39% increase in CH₄ production with radish (150 and 208 L CH₄/kg VS, respectively; p -value = 0.009) (Fig. 1; Table 3). Although the effect of forage radish content (13% vs 27% radish) on CH₄ production was not statistically compared due to differences in manure between trials, the results show that even a small quantity of radish (13% radish) added to a dairy digester results in enhanced CH₄ production.

While co-digesting forage radish cover crops in dairy digesters increased CH₄ production, the CH₄ potential was lower than values from other energy crop digestion studies (i.e., maize) but was similar to values from other sulfur-rich feedstocks. Amon et al. (2007) observed CH₄ production levels of 398 L CH₄/kg VS with maize, while Masse et al. (2010) observed 309 L CH₄/kg VS with switchgrass. Lower CH₄ production in our study (210 and 208 L CH₄/kg VS for RM1, RM2) is likely mostly due to co-digestion with manure, but could also be related to the higher sulfur content of the radish (0.6% DM) compared to the other energy crops utilized in these studies. The CH₄ potential of forage radish co-digestion was similar to other sulfur-rich biomass: seaweed (*Ulva sp.*) (148 L CH₄/kg VS),

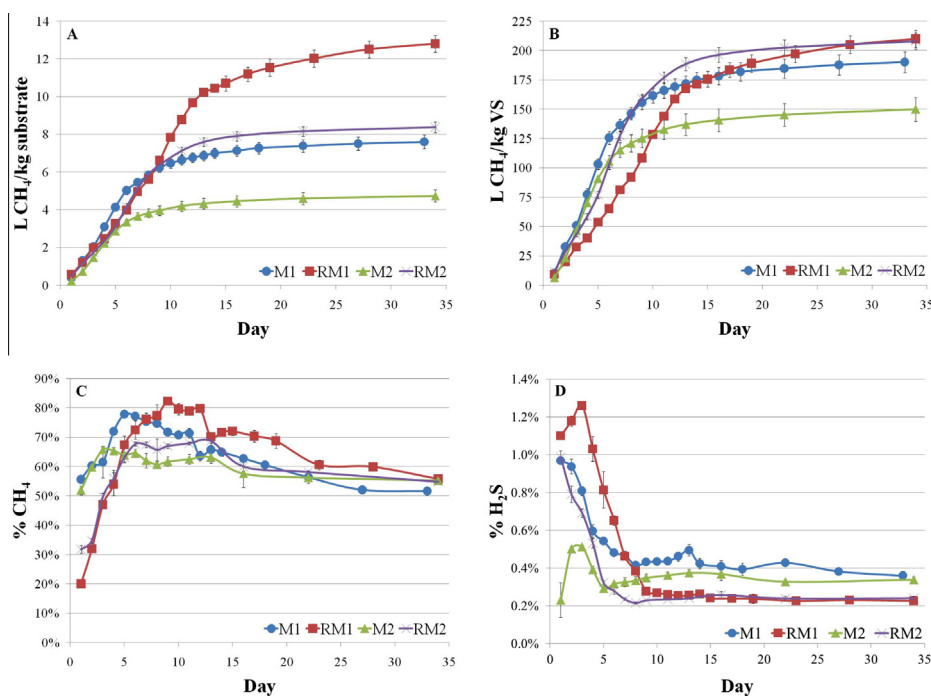


Fig. 1. Average (\pm standard error) CH₄ and H₂S production for the manure-only (M1; M2) and radish + manure digesters (RM1: 27%; RM2: 13% radish, by ww). (A) L CH₄/kg substrate added, (B) L CH₄/kg VS added, (C) percent CH₄, and (D) percent H₂S.

Table 3
Cumulative CH₄ and H₂S production represented by average (\pm standard error).

	M1	RM1	M2	RM2
L CH ₄ /kg VS	190 (9)	210 (7)	150 (10)	208 (7)
L H ₂ S/kg VS	1.71 (0.06)	2.21 (0.05)	0.91 (0.04)	1.68 (0.02)
L CH ₄ /kg substrate _{ww}	7.61 (0.35)	12.8 (0.4)	4.74 (0.32)	8.38 (0.28)
L H ₂ S/kg substrate _{ww}	0.07 (0.002)	0.14 (0.003)	0.03 (0.001)	0.07 (0.0008)

camelina (234 L CH₄/kg VS), and white mustard (223 L CH₄/kg VS) (Peu et al., 2011, 2012). Extrapolating from the pilot-scale studies, assuming no synergistic effects from co-digesting forage radish with dairy manure, digestion of 100% radish substrate would produce 515 L CH₄/kg VS. However, it is very likely that this value is overestimated, as our previous laboratory studies have shown that inoculum level (alkalinity) plays a major role in the digestion process as radish content increases (data unpublished).

3.2. Total sulfur content and H₂S production

During the first 3 days of incubation, there were elevated H₂S concentrations in the biogas produced in RM1 (1.2%), RM2 (0.8%), and M1 (0.9%), and a lower initial H₂S concentration in M2 (0.4%) (Fig. 1). H₂S production rapidly declined during incubation as CH₄ production increased, likely due to the rapid utilization of the sulfur substrate over the first 14 days. By Days 9 and 7 respectively, RM1 and RM2 had lower H₂S concentrations (0.28% and 0.23%) compared to M1 and M2 (0.43% and 0.32%), and remained below the manure-only digesters throughout the rest of the 33-day digestion period.

The sulfur content of the radish used in this study was 0.65 mg S/g radish (ww basis). Our previous laboratory experiments showed the forage radish had twice the total sulfur content of dairy manure. The range for liquid dairy manure has been cited as 0.10–0.48 mg S/g manure (ww basis) (Page et al., 2014; Bao et al., 2010). The results show that there was no difference initially in total sulfur concentration between the manure-only and radish + manure digesters (Fig. 2). It is likely that the sulfur concentrations were similar due to the relatively small proportion of radish (13% and 6% of total volume for RM1 and RM2, respectively) added to the radish + manure digesters in comparison to the proportion of inoculum and manure.

Sulfur concentrations in the digesters followed a similar pattern during both trials. In the first trial, the total sulfur content of RM1 remained constant at ~0.45 mg S/g feedstock during the first 6 days of incubation, decreased by nearly 50% to 0.24 mg/g between Days 6 and 14, and then remained relatively constant

for the remainder of the incubation period. For M1, RM2, and M2, sulfur concentrations decreased slightly during the first 6 days of digestion, decreased more steeply between Days 6 and 14, and remained stable thereafter. By Day 14, all digesters had similar total sulfur concentrations (0.18–0.22 mg S/g feedstock).

Although initial total sulfur concentrations were similar between M1 and RM1 and between M2 and RM2, the radish + manure digesters produced significantly more H₂S than the respective manure-only digesters (*p*-values = 0.009 and <0.0001 for Trial 1 and 2, respectively). This suggests that the radish substrate was more readily degradable than the manure, allowing for the sulfur substrate in the radish + manure digesters to be rapidly converted to H₂S. RM1 produced 2.21 L H₂S/kg VS in comparison to M1 (1.71 L H₂S/kg VS), while RM2 produced 1.68 L H₂S/kg VS compared to M2 (0.91 L H₂S/kg VS). With more H₂S being produced in the radish + manure digesters, CH₄ production was suppressed initially. A similar result was shown during the digestion of apple waste. In those experiments, elevated H₂S concentrations and slow increases in CH₄ production during the initial incubation illustrated that SRB activity was greater than methanogenic activity (Kafle and Kim, 2013). In that study, the authors predicted that acidification of the apple waste may have contributed to the low methanogenic activity during the initial incubation. However, in the present study, no significant acidification of the forage radish was observed as pH remained relatively constant over the entire incubation period (Fig. 2). Our results suggest that it was the sulfur content of the radish and its rapid degradation that led to significantly higher H₂S production which suppressed CH₄ production initially rather than pH causing CH₄ suppression. Similarly Kafle et al. (2014) demonstrated that the AD of another Brassica crop, Chinese cabbage waste, produced biogas with less than 50% CH₄ content during the initial days (<21 days) of incubation, with H₂S concentrations exceeding 5000 ppm. However after 21 days, the H₂S concentration gradually declined and the CH₄ concentration increased to ~80%.

The initial lag in CH₄ production observed in the radish + manure digesters could be due to a longer period of acclimatization required for digesting the radish cover crop or

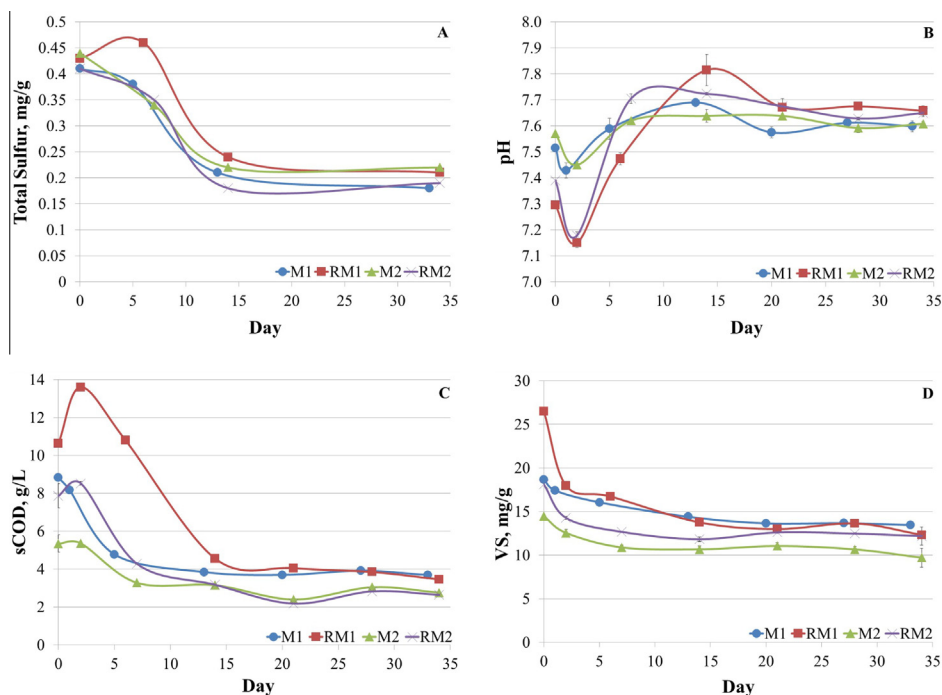


Fig. 2. Characteristics of the manure-only and radish + manure digesters during 33 days of incubation. (A) mg S/g feedstock added, (B) pH, (C) g sCOD/L feedstock added, and (D) mg VS/g feedstock added.

methanogenic inhibition by SRB. Brassica cover crops (i.e., camelina, radish fodder, mustard) have high levels of sulfur-containing glucosinolates. 30.3% of the total sulfur content in radish cover crops are glucosinolates (Peu et al., 2013). Although glucosinolate content is highly dependent on species, variety, and cultivar, radish was found to contain the highest concentration of glucosinolates (64–332 mg/100 g) relative to other crucifers such as cabbage, turnip, and kale (20–151 mg/100 g) (Ciska et al., 2000). Future studies should include a full sulfur analyses in the liquid and gas phase to further elucidate the lag phase observed. Additionally, the lag phase seen during batch digestion may not be present during continuous operation.

Although H₂S can have a corrosive effect on digestion systems, this research suggests that at peak CH₄ production co-digestion with the forage radish crop generates biogas that would not require any additional desulfurization beyond the standard practice of scrubbing the biogas from digestion of dairy manure prior to use in a CHP system. All digesters in the study had H₂S in the biogas above 0.05% (target concentration for CHP engines). On average, RM1 and RM2 had a H₂S concentration of approximately 0.20% (2000 ppm) during peak CH₄ production, with the manure-only digesters being slightly higher at 0.40% (4000 ppm) and 0.34% (3400 ppm) for M1 and M2, respectively (Fig. 1). During peak CH₄ production, the radish co-substrate lowered the H₂S concentration of the biogas below the manure-only digesters by Days 9 and 7 for Trial 1 and Trial 2, respectively, although cumulatively the radish + manure digesters produced a larger quantity of H₂S when normalized by VS addition.

Overall, CH₄ production was not severely suppressed by radish addition during this batch process and was able to reach high concentrations of CH₄ after the initial lag phase. Similarly, Peu et al. (2013) demonstrated that the digestion of Brassica crops was not severely inhibited by glucosinolate content, but H₂S production in the biogas was not directly measured in their study. Peu et al. (2012) predicted that based on total sulfur content and biogas production that digestion of radish cover crops would produce biogas containing H₂S concentration ≤ 1.0% (v:v). Although the radish species and variety were not specified and the whole plant was used as substrate, the predicted value is similar to our findings in which only the radish above-ground biomass was used for co-digestion in order to maintain the soil nutrient benefits of the cover crop and reduce harvesting labor.

3.3. pH, nutrients, and organic matter transformations

Use of forage radish as a co-digestion substrate at an ISR of 53:47 required no pH adjustments during the incubation period. All digesters remained within the optimal pH range (6.5–8.0) for mesophilic AD (Fig. 2; Table 4). Although the forage radish had an initial pH of ~4.5, adequate buffering capacity was maintained in this study for a circum-neutral digestion environment.

The addition of forage radish as a co-substrate also did not diminish the fertilizer value of the digester effluents (Table 5). TKN values for the manure-only and radish + manure digester

Table 5

Nitrogen and phosphorus characteristics of the digester effluents after 33 days of incubation.

	M1	RM1	M2	RM2
TKN (mg/L)	1750 (220)	1800 (260)	1640 (40)	1720 (40)
TKP (mg/L)	278 (32)	274 (44)	259 (10)	283 (9)
N:P	6.3:1	6.6:1	6.3:1	6.1:1

effluents were not statistically different at 1695 ± 100 and 1764 ± 118 mg/L respectively, while TKP values were also not statistically different at 269 ± 16 and 278 ± 20 mg/L, respectively. This resulted in the digester effluents having an average N:P ratio of 6.3:1, which is close to the N:P ratio required by corn grain (6:1) (Paschold et al., 2008). The effluent of both digestion systems with and without radish would be an advantageous liquid fertilizer for corn-silage based dairy farmers.

The sCOD values increased in RM1 and RM2 during the first 2 days of digestion, likely due to the initial hydrolysis of the forage radish into simple soluble compounds (Fig. 2). After the initial spike, sCOD for RM1 decreased by 10.2 g/L during the 33-day incubation period, with 90% of the reduction occurring during the first 14 days. In RM2, sCOD values were reduced by 5.9 g/L. There was less sCOD total destruction in the M1 and M2 digesters (5.1 and 2.6 g/L, respectively) due to the lower initial values. However by the end of the incubation period, the sCOD concentrations were similar between RM1 and M1, as well as between RM2 and M2, illustrating the ability of the radish + manure digesters to utilize the additional sCOD input from the radish substrate. VS reductions were similar, with 14.2 and 5.9 mg of VS removed per gram of feedstock addition for RM1 and RM2, respectively, and 5.2 and 4.8 mg/g for M1 and M2, respectively (Fig. 2).

3.4. Farm-scale analysis

Harvesting the above-ground biomass of forage radish using a rotary mower and forage chopper, yielded 7340 ± 1050 kg/acre (fresh weight) of radish shoots and above-ground roots. Based on the pilot-scale CH₄ production results for RM2 (13% radish and 87% liquid dairy manure (ww basis)) and an ISR of 53% inoculum, a 200-dairy cow farm would require a 32 m³ batch digestion system, assuming a 15% biogas headspace (Table 6). Operating the batch digester with a 30-day retention time would require the following substrates: 1630 kg (ww) of radish (corresponding to the yield from 1/4 acre) and 10,890 kg (ww) of manure, which is equivalent to the daily manure production from 200 dairy cows, assuming a daily manure production rate of 120 lbs/dairy cow (ASAE, 2003). The calculated CH₄ production from this batch system would be 105 m³/month, which corresponds to a monthly energy yield of 3.65 × 10⁶ BTU or 1070 kWh, and is 77% greater than digesting 100% dairy manure.

Due to the low pH of forage radish (4.5) and volatile fatty acid production during digestion, adequate buffering capacity is required for optimal CH₄ production when digesting radish. A high

Table 4
Characteristics of the manure-only and radish + manure mixtures before and after digestion.

	M1		RM1		M2		RM2	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
pH	7.5 (0.02)	7.6 (0.02)	7.3 (0.01)	7.7 (0.02)	7.6 (0.00)	7.6 (0.01)	7.4 (0.00)	7.7 (0.02)
sCOD (g/L)	8.83 (0.05)	3.69 (0.18)	10.6 (0.1)	3.46 (0.02)	5.34 (0.46)	2.76 (0.03)	7.87 (0.64)	2.64 (0.01)
TS (mg/g)	27.6 (0.1)	22.3 (0.4)	36.9 (0.2)	22.5 (0.1)	21.4 (0.1)	16.7 (1.5)	26.1 (0.2)	21.1 (1.5)
VS (mg/g)	18.7 (0.1)	13.4 (0.3)	26.5 (0.2)	12.3 (0.1)	14.5 (0.04)	9.7 (1.1)	18.1 (0.2)	12.2 (1.0)
Sulfur (mg/g)	0.41	0.18	0.43	0.21	0.44	0.22	0.41	0.19

Table 6

Energy and revenue per month for a 200-dairy cow farm operating a complete mix digestion system.

	Batch digester	Continuous digester
Retention time (days)	30	30
Feedstocks		
Forage radish ^a (kg _{ww})	1630	48,800
Dairy manure ^b (kg _{ww})	10,890	326,590
Inoculum (kg _{ww})	14,110	14,110
Total (kg _{ww})	26,620	389,500
Digester size ^c (m ³)	32	460
CH ₄ production ^d (m ³)	105	3150
Energy yield (BTU)	3.65E+06	1.09E+08
Energy yield (kJ)	3.85E+06	1.15E+08
Energy yield (kWh)	1070	32,050
Cover crop cost-share ^e (US\$/acre)	80	80
Radish substrate value (US\$)	20	560
Natural gas ^f (US\$/m ³)	0.43	0.43
Electricity ^g (US\$/kWh)	0.10	0.10
Co-digestion revenue		
Gas (US\$)	45	1350
Electricity (US\$)	105	3125
Total revenue		
Gas (US\$)	65	1910
Electricity (US\$)	125	3685

^a Based on 7340 kg of above-ground radish biomass/acre.

^b Assumes a daily manure production rate of 120 lbs/dairy cow.

^c Assumes a 15% volumetric headspace.

^d Based on 8.38 L CH₄/kg substrate.

^e Assumes a base payment, plus add-on incentives: cover crop planted by October 1 on a farm located in a targeted watershed on fields that previously grew corn and were fertilized in Spring with manure.

^f Based on the average price of natural gas (2013 US\$).

^g Based on average price of electricity (2013 US\$).

volume of inoculum and/or manure would likely be required, especially if operating in batch mode. Other studies have shown that high inoculum/manure loads enhanced CH₄ production when digesting Brassica crops. [Peu et al. \(2013\)](#) utilized pig slurry for radish fodder (whole plant) co-digestion (75:25, ww), whereas [Carvalho et al. \(2011\)](#) used previously digested wastewater treatment plant sludge as inoculum for oilseed radish (whole plant silage) digestion (91:9, ww), with both being higher than the ISR used in this study (53:47).

Extrapolating from the batch digestion studies to a continuously operated digestion system would require a 460 m³ digester for a 30-day retention time with manure loaded from 200 dairy cows and 1630 kg of radish daily, corresponding to seven acres of harvested radish per month. This assumes a one-time loading of 53% inoculum at start-up ([Table 6](#)). The CH₄ production from this system was calculated to be 3150 m³/month, assuming the CH₄ production rate of the batch system. The CH₄ production value assumes that the pH would remain the same in a continuous system when inoculum was only added at start-up. However, this may not occur. If fluctuations in pH occurred, the radish content could be lowered for continuous operation.

For field-scale operation, the entire daily manure load of the farm could be treated and the amount of radish harvested and added to the digester would be based on the daily manure production rate. For a new digester operation, the additional digester capacity required for radish co-digestion could be taken into account at the design phase. For an existing system, the operator has several options including decreasing in-vessel gas storage space or retention time, or storing the radish and adding a smaller percentage of radish daily in order to accommodate the existing digester size.

On a practical standpoint, our experience showed that utilizing a reduced radish loading resulted in more favorable digester oper-

ation and maintenance conditions. Reducing the radish content minimized clogging in the digester pumping system and allowed for the mechanical mixers to adequately agitate substrates without increased strain, thus reducing stratification of substrates in the digester. The smaller radish addition could also be more advantageous to the dairy farmer since less land would need to be harvested for the digestion co-substrate, resulting in reduced fuel costs associated with harvesting and transportation and less labor and energy would be required for harvesting and mechanically reducing the particle size of the radish.

A possible scenario for a corn-silage based dairy farmer utilizing a continuous complete mix digester would consist of the following. The forage radish cover crop would be planted in August immediately after corn harvest. Starting November, the forage radish cover crop would be harvested for co-digestion substrate weekly. Due to the consistency of the above-ground radish biomass after mowing and forage chopping, it is more ideal to harvest the radish in small batches. The colder temperatures should allow for the harvested radish to be stored outdoors. The ambient temperature will dictate if weekly radish harvesting can continue through December and how many additional acres could be harvested for continual loading in December and January. Typically in December or January, the forage radish cover crop will winter-kill after several consecutive nights with temperatures below -6 °C. The forage radish cover crop that will be utilized as co-substrate should be harvested prior to any predicted hard frost.

Through cost-share programs, farmers can receive grants for planting cover crops. Depending upon the farming practice, the average base payment is \$45/acre. Using highly valued planting practices, such as planting in fields that previously grew corn or were fertilized with manure in the springtime can increase the payment to \$80/acre ([MDA, 2014](#)). Utilizing the above-ground radish biomass as co-substrate could also increase farm revenue through biogas and electrical generation. Based on CH₄ production values from the batch and continuous digestion systems on a 200-dairy cow farm and the 2013 average price of natural gas (0.43 US\$/m³) and electricity (0.10 US\$/kWh) ([EIA, 2014](#)), co-digesting with radish could provide additional revenue up to \$45 and \$1350/month, respectively, for natural gas and \$105 and \$3125/month, respectively, for electricity.

4. Conclusions

Increased renewable energy production can be realized through co-digestion with forage radish cover crops in dairy manure digesters. Although cumulative H₂S production in the radish + manure digesters was significantly higher than the respective manure-only digester, H₂S production in the radish + manure digesters rapidly declined, resulting in higher levels of CH₄ (>67%) and lower levels of H₂S (0.20%) in the biogas compared to the manure-only digesters during peak biogas production. For a 200-dairy cow farm, a continuous complete mix digester (30-day retention time) would require seven acres of radish and has the potential to produce 3150 m³ CH₄/month through co-digestion.

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References

- AgSTAR, 2013. Accomplishments. Page last modified June 30, 2014. Available from <<http://www.epa.gov/agstar/about-us/accomplish.html>> (accessed Sept 1, 2014).
- Al Seadi, T., Rutz, D., Prassl, H., Kottner, M., Finsterwalder, T., Volk, S., Janseen, R., 2008. Biogas Handbook. University of Southern Denmark, Esbjerg.
- Alvarez, R., Liden, G., 2008. Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. *Renew. Energy* 33 (4), 726–734.
- Amon, T., Amon, B., Kryvoruchko, V., Machmüller, A., Hopfner-Sixt, K., Bodiroza, V., Hrbek, R., Friedel, J., Pötsch, E., Wagentristl, H., Schreiner, M., Zollitsch, W., 2007. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresour. Technol.* 98 (17), 3204–3212.
- APHA (American Public Health Association), 2005. In: Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., Franson, M.A.H. (Eds.), *Standard Methods for the Examination of Water and Wastewater*, 21st ed. American Public Health Association, Washington, DC.
- ASAE, 2003. Manure Production and Characteristics. ASAE Standard D384.1Feb03.
- Bao, Y., Guan, L., Zhou, Q., Wang, H., Yan, L., 2010. Various sulphur fractions changes during different manure composting. *Bioresour. Technol.* 101 (20), 7841–7848.
- Braun, R., Weiland, P., Wellinger, A., 2009. Biogas from energy crop digestion. IEA Bioenergy, Task 37. Available from <http://biogasmax.co.uk/media/iea_1_biogas_energy_crop_007962900_1434_30032010.pdf> (accessed Aug 27, 2014).
- Bruni, E., Jensen, A.P., Pedersen, E.S., Angelidaki, I., 2010. Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. *Appl. Energy* 87 (7), 2212–2217.
- Carvalho, L., Di Berardino, S., Duarte, E., 2011. Biogas production from Mediterranean crop silages. In: *Proceedings Sardinia 2011. Thirteenth International Waste Management and Landfill Symposium*. Cagliari, Italy, October 3–7, 2011.
- Ciska, E., Martyniak-Przybyszewska, B., Kozłowska, H., 2000. Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. *J. Agric. Food Chem.* 48 (7), 2862–2867.
- Deublein, D., Steinhauser, A., 2011. *Biogas from Waste and Renewable Resources*, second ed. Wiley-VCH, Weinheim, Germany.
- EIA, 2014. Independent statistics and analysis. U.S. Energy Information Administration. Available from <<http://www.eia.gov/dnav/ng/hist/n3010us3m.htm>> (accessed Aug 15, 2014).
- El-Mashad, H.M., Zhang, R., 2007. Co-digestion of food waste and dairy manure for biogas production. *Trans. ASABE* 50 (5), 1815–1821.
- El-Mashad, H.M., Zhang, R., 2010. Biogas production from co-digestion of dairy manure and food waste. *Bioresour. Technol.* 101 (11), 4021–4028.
- Gruver, J., Weil, R., White, C., Lawley, Y., 2014. Radishes: a new cover crop for organic farming systems. Available from <<http://www.extension.org/pages/64400/radishes-a-new-cover-crop-for-organic-farming-systems#U6Lj3HZ2Ny0>> (accessed Aug 19, 2014).
- Kafle, G.K., Kim, S.H., 2013. Anaerobic treatment of apple waste with swine manure for biogas production: batch and continuous operation. *Appl. Energy* 103, 61–72.
- Kafle, G.K., Bhattarai, S., Kim, S.H., Chen, L., 2014. Effect of feed to microbe ratios on anaerobic digestion of Chinese cabbage waste under mesophilic and thermophilic conditions: biogas potential and kinetic study. *J. Environ. Manage.* 133, 293–301.
- Khanal, S.K., Huang, J., 2003. ORP-based oxygenation for sulfide control in anaerobic treatment of high-sulfate wastewater. *Water Res.* 37, 2053–2062.
- Klavon, K.H., Lansing, S., Moss, A., Mulbry, W., Felton, G., 2013. Economic analysis of small-scale agricultural digesters in the United States. *Biomass Bioenergy* 54, 36–45.
- Lansing, S., Martin, J.F., Botero, R.B., da Silva, T.N., da Silva, E.D., 2010. Methane production in low-cost, unheated, plug-flow digesters treating swine manure and used cooking grease. *Bioresour. Technol.* 101 (12), 4362–4370.
- Liu, Y., Whitman, W.B., 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Ann. N. Y. Acad. Sci.* 1125 (1), 171–189.
- Lounsbury, N.P., 2013. *Spring Seedbed Characteristics After Winterkilled Cover Crops* (MS thesis). University of Maryland, College Park.
- Madigan, M.T., Martinko, J.M., Stahl, D.A., Clark, D.P., 2012. *Brock Biology of Microorganisms*, 13th ed. Benjamin Cummings, San Francisco, CA.
- Masse, D., Gilbert, Y., Savoie, P., Belanger, G., Parent, G., Babineau, D., 2010. Methane yield from switchgrass harvested at different stages of development in Eastern Canada. *Bioresour. Technol.* 101 (24), 9536–9541.
- MDA, 2014. Maryland agricultural water quality cost-share program. Maryland Department of Agriculture. Available from <http://mda.maryland.gov/resource_conservation/Pages/cover_crop.aspx> (accessed Aug 30, 2014).
- Page, L.H., Ni, J., Heber, A.J., Mosier, N.S., Liu, X., Joo, H., Ndegwa, P.M., Harrison, J.H., 2014. Characteristics of volatile fatty acids in stored dairy manure before and after anaerobic digestion. *Biosyst. Eng.* 118, 16–28.
- Paschold, J.S., Wienhold, B.J., McCallister, D.L., Ferguson, R.B., 2008. Crop nitrogen and phosphorus utilization following application of slurry from swine fed traditional or low phytate corn diets. *Agron. J.* 100 (4), 997–1004.
- Peu, P., Sassi, J.F., Girault, R., Picard, S., Saint-Cast, P., Beline, F., Dabert, P., 2011. Sulphur fate and anaerobic biodegradation potential during co-digestion of seaweed biomass (*Ulva* sp.) with pig slurry. *Bioresour. Technol.* 102, 10794–10802.
- Peu, P., Picard, S., Diara, A., Girault, R., Beline, F., Bridoux, G., Dabert, P., 2012. Prediction of hydrogen sulphide production during anaerobic digestion of organic substrates. *Bioresour. Technol.* 121, 419–424.
- Peu, P., Picard, S., Girault, R., Labreuche, J., Beline, F., Dabert, P., 2013. Catch crops for agricultural biogas production, case study for Brassicaceae sp. In: *Proceedings 13th World Congress on Anaerobic Digestion*, at Santiago de Compostela, Spain (IWA-11151), June 25–28, 2013.
- SARE, 2007. *Managing Cover Crops Profitably*, third ed. Sustainable Agriculture Network, Beltsville, Maryland.
- Umetsu, K., Yamazaki, S., Kishimoto, T., Takahashi, J., Shibata, Y., Zhang, C., Misaki, T., Hamamoto, O., Ihara, I., Komiyama, M., 2006. Anaerobic co-digestion of dairy manure and sugar beets. *Int. Congr. Ser.* 1293, 307–310.
- Wang, Q., Thompson, E., Parsons, R., Rogers, G., Dunn, D., 2011. Economic feasibility of converting cow manure to electricity: a case study of the CVPS cow power program in Vermont. *J. Dairy Sci.* 94, 4937–4949.