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Keywords
Anaerobic digestion, Aqueous ammonia soaking, Biochemical methane potential, Biogas, Biomass pretreatment, BMP

Comments
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Abstract. *This article presents the biochemical methane potential (BMP) results from the anaerobic digestion (AD) of switchgrass. Triplicate BMP assays were performed on: untreated switchgrass, aqueous ammonia soaking (AAS) pretreated switchgrass (soaked in 29.5% reagent-grade aqueous ammonia at 5 L kg^{-1} switchgrass for 5 d), and AAS-pretreated switchgrass plus cellulytic enzymes at 12.5, 25, 62.5, and 125 filter paper units (FPU) enzyme g^{-1} volatile solids (VS). Biogas production and biogas methane content were measured daily in all treatments for 21 d. Both biogas and corrected methane production varied significantly among treatments, especially during the first 7 d of the BMP period. Total methane production at 21 d was corrected for enzyme degradation, and methane yields ranged from 0.15 to 0.36 m^3 CH_4 kg^{-1} VS. We compared the corrected energy yield of biogas from switchgrass to prior reports of the energy yield of ethanol from switchgrass via simultaneous saccharification and fermentation (SSF). The AD of AAS-pretreated switchgrass at the highest enzyme loading rates resulted in a 120% increase in energy extracted as compared to AAS-pretreated switchgrass converted to ethanol via SSF. Overall, the addition of enzymes to AAS-pretreated switchgrass greatly accelerated the rate of methane production over the untreated switchgrass and AAS-pretreated switchgrass without enzymes. However, the process economics are not clear, and additional work is needed to determine whether pretreating switchgrass with aqueous ammonia and/or enzymes before AD is economically advantageous.*

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Current schemes for biofuel production generally focus on liquid transportation fuels such as ethanol and biodiesel. Each has its own challenges, ethanol in part because of the energy-intensive distillation step (Ragauskas et al., 2006) and biodiesel because of its relatively low energy per unit cropped area (Pimentel and Patzek, 2005). A biofuel derived from a high-yielding lignocellulosic feedstock that does not require significant processing energy inputs is an attractive target. One alternative is biogas, which self-separates from the aqueous reactor contents and is already used as a transportation fuel in northern Europe (Svensson et al., 2006; Auer et al., 2006). Sweden, the largest producer of biogas, uses upgraded biogas as a vehicle fuel in buses, rail, distribution trucks, and passenger cars, as well as fuel for heat or combined heat and power (Auer et al., 2006; Lantz et al., 2007). Biogas, composed mainly of methane and carbon dioxide, is produced through the anaerobic digestion (AD) of a variety of biomass substrates including lignocellulosic material. In addition to the low energy investment required to produce biogas from biomass, methane is an attractive vehicle fuel from an end-use air-quality standpoint: one commercially available compressed-natural-gas powered vehicle is certified as a partial-zero emission vehicle (Ridlington and Davis, 2005).

Lignocellulosic material is the most abundant organic resource on earth and is thus a promising raw material for bioenergy production (Lynd and Wang, 2004). Extensive reviews of AD of various feedstocks, including lignocellulosic material for methane production, have been published previously (Gunaseelan, 1997; Chynoweth et al., 1993; Smith et al., 1992). Lignocellulosic feedstocks, such as corn stover and wheat straw, were identified as substrates with excellent methane potential, yielding 0.360 to 0.383 m^3 CH_4 kg^{-1} volatile solids (VS) added during 60 d biochemical methane potential (BMP) trials (Gunaseelan, 1997). BMP trials of switchgrass completed by Labatut and Scott (2008) yielded approximately 0.12 m^3 CH_4 kg^{-1} VS and corn silage yielded 0.3 m^3 CH_4 kg^{-1} VS during 60 d digestions. Of 30 substrates tested by Labatut and Scott (2008), switchgrass had the lowest gas production and achieved only 29% of the theoretical maximum yield based on the stoichiometric relationship between COD and methane production, suggesting significant potential to improve the digestion of this recalcitrant biomass.

The BMP assay was developed as a standardized method to determine the anaerobic degradability and the potential methane yield during anaerobic methanogenic fermentation of organic material (Speece, 1996). A modified method

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based on the procedure outlined by Owen et al. (1979) involves batch incubation of substrates under conditions ideal for anaerobic decomposition, to evaluate digestibility and biogas production. This BMP procedure provides a valuable and inexpensive method to determine the potential extent and rate of conversion of candidate feedstocks.

Lignin, a major constituent of plants, hinders cellulose decomposition under anaerobic conditions in lignocellulosic biomass (Stinson and Ham, 1995), with methane yields inversely related to lignin content (Smith et al., 1992). Pretreatment of lignocellulosic material modifies the lignin bonds and enhances the biodegradability by freeing cellulose and hemicellulose, possibly increasing biogas production (Yadvika et al., 2004). Alkaline pretreatment at ambient temperature has been proposed as a chemical process compatible with AD because of the desirable high pH level (Neves et al., 2006). In a 50 d experiment, AD of alkali-pretreated wheat straw produced 37% to 100% more methane than the untreated wheat straw (Pavlostathis and Gossett, 1985). He et al. (2008) found that pretreating rice straw with 6% sodium hydroxide increased 21 d biogas yield by 27% to 65%. However, pretreating winter rye, oilseed rape, and faba beans with Na_2CO_3 at 195°C and 1200 kPa for 15 min failed to significantly increase methane production in a 50 d trial, possibly due to inhibitors produced during the high-temperature, high-pressure pretreatment (Petersson et al., 2007). Low-temperature, low-pressure aqueous ammonia soaking (AAS) pretreatment appears to be an attractive pretreatment method for AD.

The AD of lignocellulosic biomass is a relatively slow biological process, generally accomplished at hydraulic retention times (HRT) of 30 to 50 d. In contrast, the AD of simple substrates can be accomplished at HRTs ranging from less than one day to 3 d for readily degradable food wastes (Yadvika et al., 2004; Moody and Raman, 2001). Cellulosic material is converted to simple substrates by hydrolysis, which is the rate-determining step in the conversion process of lignocellulosic material (Adney et al., 1991). Accelerating hydrolysis with a combination of pretreatment and added hydrolytic enzymes (as opposed to the endogenous hydrolytic enzymes produced by the AD microbial consortia, e.g., Lynd et al., 2002) during AD can shorten the HRT, allowing for smaller reactor volumes, and possibly improving overall process economics. Accordingly, the objective of this study was to examine the effect of AAS pretreatment, with and without added cellulase, on the AD of switchgrass. This was done by determining and comparing daily biogas production, methane content of biogas, and methane yields of the treatments. Energy yields of the AD process were compared to the energy yield of ethanol production from the same AAS-pretreated switchgrass.

Materials and Methods

Raw Materials

Switchgrass was collected from mature, 4-year-old stands of Cave-in-Rock cultivar in mid-October 2007 at the Iowa State University Agronomy and Agricultural Engineering Farm near Ames, Iowa (42° 00′ N, 93° 50′ W; elevation 341 m above sea level). The stand was established in late summer and autumn of 2003 and was fertilized at 140 kg N ha^{-1} as ammonium nitrate. Switchgrass was harvested above a 5 cm height following a killing frost. Dry switchgrass was ground to a size of 5 to 6 mm at the Biomass Energy Conversion Center (BECON, Nevada, Iowa) using a hammer mill grinder (model 400430, Art's Way, Armstrong, Iowa). Composition of the switchgrass was determined by the Iowa State University Department of Agronomy using the ANKOM method (ANKOM Technology Corp., Fairport, N.Y.) as described by Vogel et al. (1999). Klason lignin was determined as described by Crawford and Pometto (1988), slightly modified by Isci et al. (2007). Untreated switchgrass contained 41% cellulose, 32% hemicellulose, 7% acid detergent lignin, 19% Klason lignin, and 0.7% ash on a dry basis.

Pretreatment

Based on previous work by our group (Isci et al., 2007), 40 g of dry switchgrass was soaked in reagent-grade 29.5 wt% aqueous ammonium hydroxide (Fisher Scientific) in 1.0 L high-density polyethylene bottles at room temperature without agitation for 5 d. Following pretreatment, the biomass was washed *in situ* with 12 L of deionized (DI) water using the custom fluidized bed-biomass washing system (Isci et al., 2007). Aqueous ammonia soaking pretreatment removed an average of 35% of Klason lignin and 41% hemicellulose, resulting in approximately 56% cellulose in the pretreated switchgrass.

Enzyme

To be consistent with previous switchgrass-to-fuel studies by our group (Isci et al., 2007), Spezyme CP cellulase enzyme (Lot No. 301-05330-206, Genencor, Palo Alto, Cal.) was selected for this study. Cellulase enzyme activity was determined by the reducing sugar method according to Adney and Baker (1996). Activity was 55 filter paper units (FPU) mL^{-1} enzyme, and the chemical oxygen demand (COD) was determined to be 430 mg COD mL^{-1} enzyme.

Treatments

Eight treatments were evaluated, as listed in table 1. The untreated switchgrass was a baseline and enabled comparison to previous literature, while the mixed pentose/hexose control allowed assessment of the microbial community's ability to handle these hydrolysis by-products. The AAS-pretreated switchgrass was examined without enzyme and at four non-zero enzyme loading rates ranging 10-fold. An inoculum-to-substrate ratio of 1:2 (VS basis) was used in this study following Labatut and Scott (2008).

Table 1. List of treatments (AAS = aqueous ammonia soaking, FPU = filter paper units, VS = volatile solids).

Treatment	Substrate
1	Untreated switchgrass
2	AAS-pretreated switchgrass
3	AAS-pretreated switchgrass + 0.25 mL enzyme g^{-1} VS (12.5 FPU g^{-1} VS)
4	AAS-pretreated switchgrass + 0.5 mL enzyme g^{-1} VS (25 FPU g^{-1} VS)
5	AAS-pretreated switchgrass + 1.25 mL enzyme g^{-1} VS (62.5 FPU g^{-1} VS)
6	AAS-pretreated switchgrass + 2.5 mL enzyme g^{-1} VS (125 FPU g^{-1} VS)
7	60/40 glucose/xylose mixture
8	Inoculant control

BMP Assay

An aliquot of substrate was added to a 250 mL serum bottle along with 83 mL of inoculum and basal medium to equate the volume to approximately 200 mL. The substrate mass was such that the inoculum-to-substrate VS ratio was 1:2. Inoculum was obtained from a 60 L mesophilic (35˚C) continuous stirred tank reactor (CSTR), fed daily with basal medium and high-protein dog food at a loading rated of 2 g VS L^{-1} d^{-1} (Wu-Haan et al., 2008; Bishop et al., 2009). The inoculum concentration was 0.0024 g L^{-1} VS. The headspace in the serum bottle was purged with 30% CO_2 in 70% N_2 at a flow rate of approximately 0.5 L min^{-1} for 5 min and then sealed. The serum bottles were then placed in a shaker rotating at approximately 150 rpm and incubated at 35˚C (Wu-Haan et al., 2008). Each treatment was performed in triplicate.

Each day, the vials were depressurized, at ambient conditions, and biogas was collected by inserting a hypodermic needle connected to a 50 mL welted and graduated gas collection syringe through the serum cap. The biogas composition was measured daily using a nondispersive infrared sensor, the NDIR-CH_4 gas-analyzer (model 08/003, Institute of Agricultural Process Engineering, University of Kiel, Germany). Calibration with 60% CH_4 in 40% CO_2 and 30% CO_2 in 70% N_2 for 3 min at 0.3 to 0.4 L min^{-1} was performed weekly, and control checks with 60% CH_4 in CO_2 were performed prior to daily measurement. Reported results are average values of the triplicate samples.

Results and Discussion

Daily biogas production varied significantly between treatments (fig. 1). On day one, the sugar standard produced the most biogas, more than 90 mL, presumably due to the availability of simple sugars utilized by the microbial population for immediate digestion. After 2 d, the two high-enzyme treatments produced 75 and 100 mL of biogas, respectively (fig. 1). It appears that the hydrolytic enzyme addition increased hydrolysis rates, yielding significantly more methane than the other treatments. At 2 d, the biogas production rates peaked in all treatments. Peak gas production varied directly with enzyme loading level, with even the no-enzyme AAS-pretreated switchgrass producing twice as much biogas as the untreated switchgrass. Following 6 d of incubation, the biogas production in all treatments dropped below 20 mL d^{-1} and remained at low levels for the remainder of the study. Variability within treatments was modest: less than 8% of the daily biogas production data had a coefficient of variance greater than 25%, the majority of which were from the low-yielding untreated and no-enzyme AAS-pretreated switchgrass samples.

Biogas composition varied significantly during the first 12 days of incubation (fig. 2) but stabilized at 40% to 58% methane on day 12. Biogas from the two high-enzyme treatments and the sugar control reached the highest methane concentrations (50% to 58%), which is within the expected range of methane content (50% to 70%) for biogas produced from carbohydrate-rich feedstocks (Speece, 1996). These treatments with high steady-state methane content were also those with the most rapid rise in methane content (fig. 2). As with the biogas production data, variability of composition within treatments was modest: 6.5% of the biogas composition data set had a coefficient of variance greater than 5%.

Cumulative methane yield, determined from daily biogas production and methane content data, is shown in figure 3. The cumulative methane yield at 21 d ranged from 0.16 to 0.49 m^3 CH_4 kg^{-1} VS, corresponding to 20% to 98% of theoretical production based on the energy content of switchgrass. As shown in figure 2, the methane content reported for the inoculum on days 3 and 9 of the experiment is zero even though biogas was produced. This is because the volumes produced were insufficient for proper operation of the methane analyzer, which required at least 10 mL to give accurate readings. As expected, the AAS-pretreated material produced significantly more methane than the untreated switchgrass, presumably due to the breaking and removal of lignin by the pretreatment. Based on prior work by our group (Isci et al., 2007), an estimated 35% of the lignin was removed

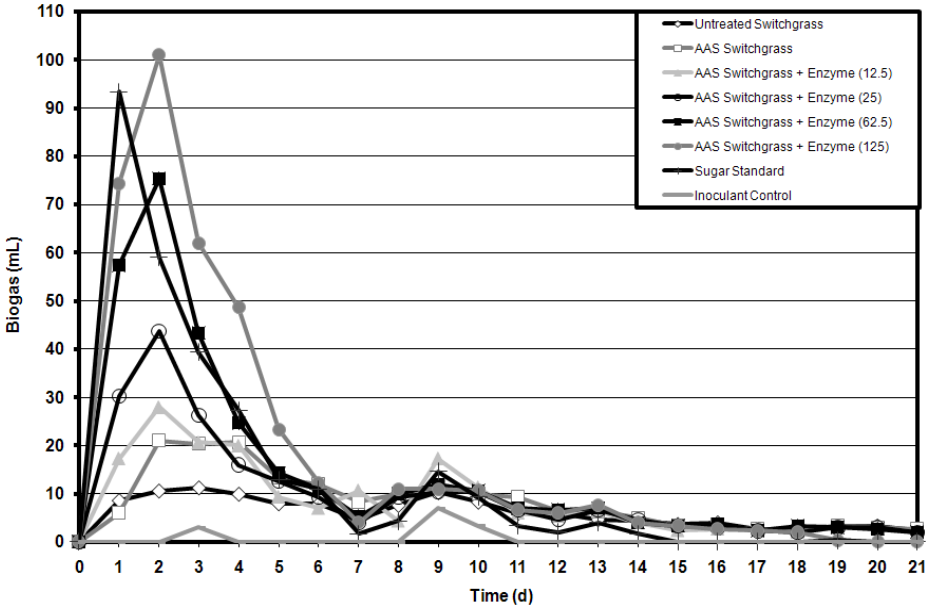

Figure 1. Daily biogas production (mL) obtained for each treatment as outlined in table 1 (*n* = 3).

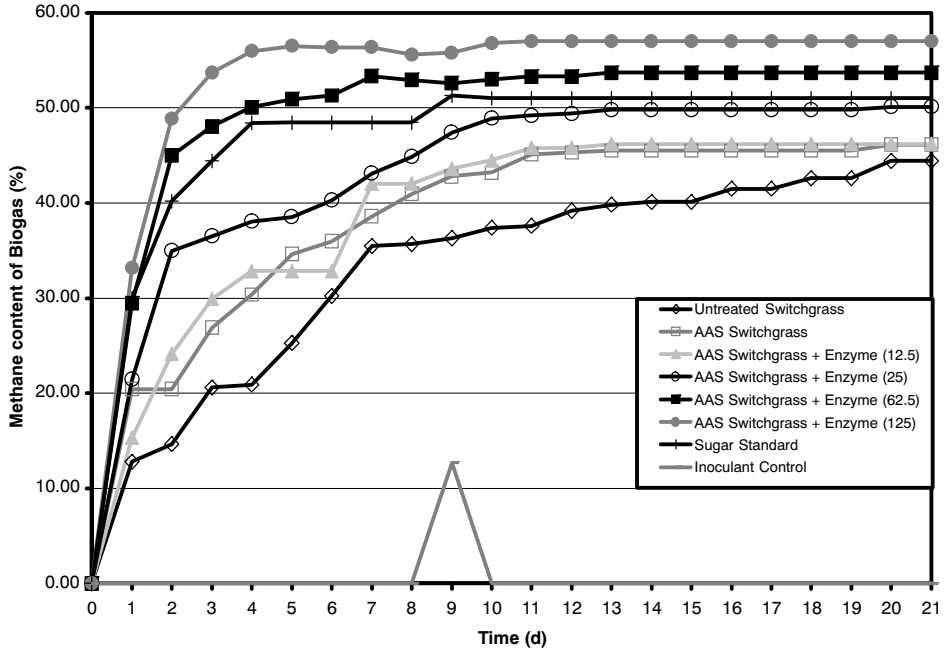

Figure 2. Methane composition of biogas (%) obtained for each treatment as outlined in table 1 (n = 3).

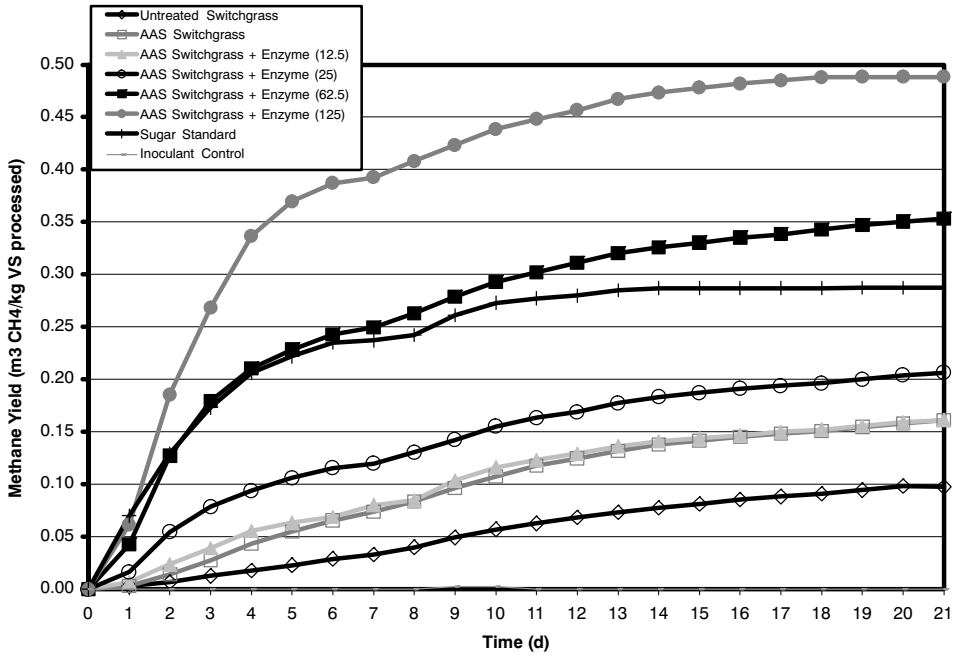

Figure 3. Methane yield (m^3 CH_4 kg^{-1} VS) during the first 21 d obtained for each treatment as outline in table 1. Note that no correction for the biogas that could be produced from the degradation of the enzyme solution is included here (n = 3).

during AAS pretreatment. This in turn freed the cellulose and hemicellulose and made them more readily available to enzymes and to microorganisms for hydrolysis and digestion. The methane yield from the lowest enzyme loading treatment of AAS-pretreated switchgrass with 12.5 FPU g^{-1} VS was not significantly different from the untreated switchgrass, suggesting that the enzyme loading was too low to hydrolyze the cellulose effectively.

This result contrasts with our experiences with low-enzyme loading ethanol fermentations (Isci et al., 2007) and suggests that significant enzyme inhibition and degradation may be occurring in the AD process. Enzymatic inhibition could be reduced in the following ways: (1) by incrementally adding enzyme, (2) by hydrolyzing biomass for 1 d prior to AD, or (3) by selecting hydrolytic enzymes better suited to AD conditions (e.g., elevated pH) (Isci et al., 2007).

At 21 d, the 25 FPU g^{-1} VS treatment produced 40% of theoretical yield based on switchgrass energy content, while the 62.5 FPU g^{-1} VS treatment reached 70% and the 125 FPU g^{-1} VS treatment reached nearly 98%. Near-optimal yield suggests that degradation of the added enzyme, a potential food source, could be contributing to biogas yield. This is not accounted for in figure 3; however, it is addressed later.

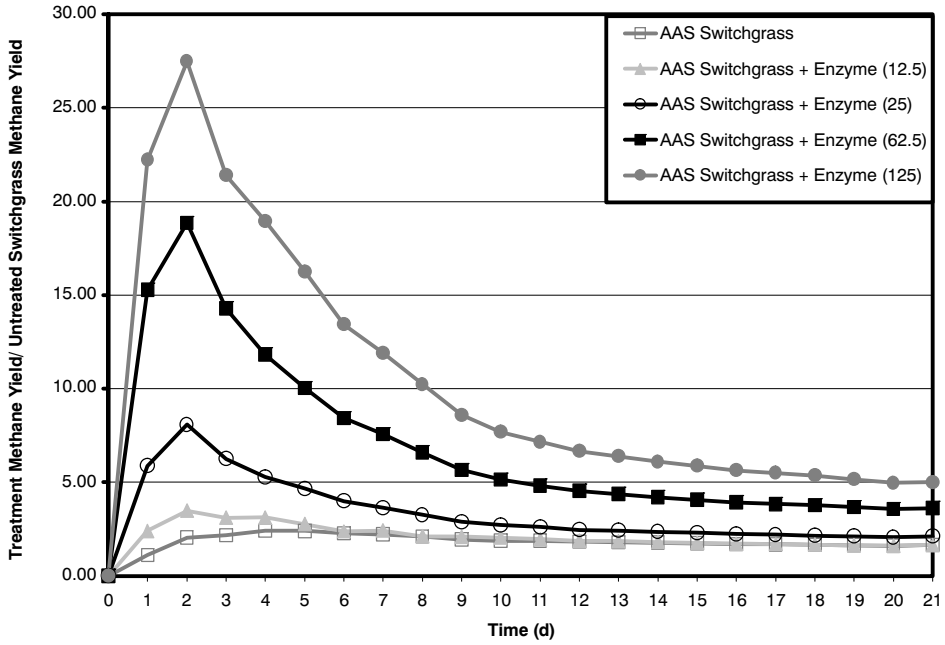

Figure 4. Ratio of methane yield (treatment methane yield/untreated switchgrass methane yield) (n = 3).

To better visualize the temporal variation in benefits, figure 4 displays a ratio of methane yield as compared with the untreated switchgrass for each treatment, on a daily basis. After 2 d, the 62.5 and 125 FPU g^{-1} VS treatments produced 18 and 27 times more methane, respectively, than the untreated switchgrass. The various pretreatments stabilized after 10 d, producing between 2 and 7 times more methane than the untreated switchgrass. Although the dramatic differences between treatments seen early in figure 4 decrease over time, they never disappear completely.

After 7 d of incubation, the treatments were compared to determine how each treatment increased the methane rate of production. Aqueous ammonia soaked switchgrass yielded 2.24 times more methane than untreated switchgrass. With the addition of enzymes, methane production increased yield by a factor of 2 to 11.9 compared to untreated switchgrass. Doubling the enzyme load from 12.5 to 25 FPU g^{-1} VS increased methane yield by 50%, while doubling the load at high doses, from 62.5 to 125 FPU g^{-1} VS increased methane yield by 57%. Overall, the 10-fold increase from the low to high enzyme loading increased the rate of methane production by a factor of 4.9. After 21 d of incubation, AAS-pretreated switchgrass yielded 1.66 times more methane than untreated switchgrass. This increase was less than that at 7 days, perhaps reflecting the ability of endogenous enzyme systems to dismantle the lignocellulosic feedstock after sufficient time. Adding enzymes increased methane production yield from 1.66 to nearly 5 times the untreated switchgrass. Doubling the enzyme load at the low doses increased methane yield by 28%, while doubling the load at high doses increased methane yield by 38%.

Figure 5 depicts the gross energy yield (MJ kg^{-1} dry-basis switchgrass added) at 2, 7, 14, and 21 d. The reference line at 7.0 MJ kg^{-1} switchgrass represents the maximum gross fuel energy yield observed from the SSF of AAS-pretreated switchgrass (1:5 solids:liquid ratio for 5 d with an enzyme loading rate of 77 FPU g^{-1} and 3% cellulose) in previous work by our group (Isci et al., 2007). Furthermore, the complete conversion of hydrolyzed cellulose and hemicellulose to ethanol would yield 11.7 MJ kg^{-1} switchgrass. Energy yields associated with AAS-pretreated switchgrass plus enzyme conditions were adjusted based on a first approximation of enzyme protein content of 116 mg mL^{-1} for Spezyme CP (Coward-Kelley et al., 2002). The 16.8 MJ kg^{-1} energy content of the protein was used to adjust energy yields of AAS-pretreated switchgrass plus enzyme by 0.195 to 1.95 MJ, depending on the enzyme loading. This assumed that all energy available in the enzyme was used during the AD. At day 2, the standard duration of an SSF experiment, none of the biogas systems produced as much energy as the ethanol fermentation. However, at longer retention times and high enzyme loadings, significantly more energy was produced by AD, with the highest enzyme loading system producing 15.5 MJ kg^{-1} switchgrass after 14 d, nearly 2.5 times more than the C6-utilizing ethanol system, but at a much longer retention time.

The results show that a significant amount of energy can be harvested from AAS-pretreated switchgrass and AAS-pretreated switchgrass with enzyme, as compared to untreated switchgrass. However, the effectiveness of any pretreatment and addition of hydrolytic enzymes must be balanced against the cost of these additions.

Enzymes are critical in converting lignocellulosic biomass to fuels and chemicals, but the high cost of these enzymes presents a significant barrier in the commercialization of biofuel technologies. It can be estimated, that at current rates, enzyme will cost approximately $30 per metric ton of switchgrass, in addition to feedstock cost (Merino and Cherry, 2007; Ritter, 2008). Based on the energy payback ($ Mg^{-1} switchgrass) calculated from the experimental results, using anaerobic digestion with enzymes and switchgrass would not be economically viable. However, extensive research efforts are underway to reduce the cost of enzymes by up to 50%, which would improve the economics for enzyme addition. Without system optimization and scale-up of this bench-scale process, an economic analysis is premature,

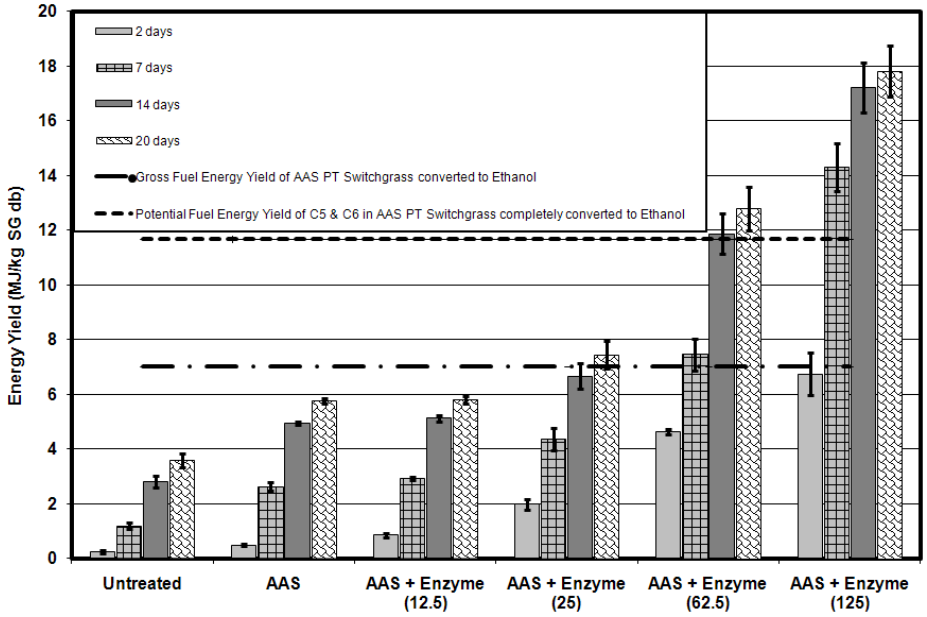

Figure 5. Gross energy yield of treatments compared to gross energy yield of AAS-pretreated switchgrass converted to ethanol via SSF at 2, 7, 14, and 20 d corrected assuming for energy yield of protein in enzyme (*n* = 3).

but looks unfavorable until reductions in enzyme costs are realized.

Conclusions

Aqueous ammonia steeping is a relatively simple delignification pretreatment method for biomass that significantly increases biogas energy production from the anaerobic digestion of switchgrass. After 21 d of incubation, AAS-pretreated switchgrass produced 65% more methane than the untreated switchgrass. The addition of sufficient commercially available hydrolytic enzymes greatly increased biogas yields, methane concentration, and total methane yields. At 21 d, the lowest enzyme treatment (12.5 FPU g^{-1} VS) was not significantly different from the non-enzyme AAS-pretreated switchgrass. However, relative to the no-enzyme treatment, the AAS-pretreated switchgrass with 25, 62.5, and 125 FPU g^{-1} produced 130%, 227%, and 325% more methane, respectively. AAS-pretreated switchgrass at 125 FPU g^{-1} VS reached 98% of theoretical methane yield on a switchgrass energy content basis and 50% more energy yield than available from the carbohydrate fraction of the switchgrass. At the highest enzyme loading, gross energy production from AD was well over twice the gross energy production from ethanol fermentation of the same material, and this energy difference would be expected to grow when the separation energy requirements of ethanol are included. However, the AD approach does not produce a liquid transportation fuel, and it requires significantly longer retention times (21 d vs. ~2 d) to extract this excess energy. Other factors, such as residue use and fuel value, must be considered in determining the merits of this AD approach relative to cellulosic ethanol systems. However, these preliminary results suggest that further work on the enzyme-enhanced AD of pretreated biomass is justified.

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