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Evaluating the Impacts of *Phragmites australis* Pretreatment Methods on Biogas and Methane

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Abstract

Phragmites australis *is an invasive species of large perennial grass in Wisconsin. One promising option for mitigation is to harvest* Phragmites australis *and convert the biomass to energy through biogas production by anaerobic digestion. This study evaluated the impact of several pretreatment methods on the amount of biogas and methane that can be produced from anaerobically digesting* Phragmites australis*. The pretreatment methods evaluated, included 1) no pretreatment, 2) mechanical—cutting/shredding, 3) mechanical—grinding, 4) thermal, 5) ultrasound, 6) alkali, 7) acid, 8) aerobic, and 9) anaerobic. The 30-day biomethane potential assay was used to evaluate the production of biogas. Methane content was measured on Day 30 using a gas chromatographer with thermal conductivity detector. The quantity of methane produced was calculated by multiplying the maximum biogas produced by the methane content. The results showed that shredding, grinding, thermal, ultrasound aerobic, and anaerobic pretreatments resulted in a significant increase in overall biogas production. While no pretreatment affected the methane content in the biogas, shredding, grinding, thermal, aerobic, and anaerobic pretreatments resulted in an increase in methane production. Thus, utilizing these pretreatment methods prior to anaerobic digestion may increase the energy obtained from* Phragmites australis*, which could provide some cost savings and make this mitigation strategy more economically feasible.*

Keywords: Pragmites australis*, biogas, methane, pretreatment, digestion*

Introduction

Phragmites australis is an invasive species of large perennial grass that is invading and outcompeting native species in low-lying, low-drainage areas, including roadside ditches and wetlands in Wisconsin. Unfortunately, as *Phragmites australis* takes over an ecosystem, the ecosystem becomes homogenized and the natural and economic value of the ecosystem, including plant biodiversity, fish and wildlife habitat quality, and biogeochemical cycle disruptions, is diminished (Bertness et al., 2002; Chambers et al., 1999; Findlay et al., 2003; Gratton & Denno, 2006). As a result, much research has focused on mitigation methods for controlling *Phragmites australis*, including; 1) tilling, 2) mowing or cutting, 3)

using managed fire, and 4) using herbicides. However, these options are often undesirable because of the high time and resource costs and because of perceived or real environmental concerns with the method (i.e., chemical usage). Fire use has also been limited because it has the potential to increase *Phragmites australis* density by removing plant litter. One promising option for addressing these economic concerns is to harvest *Phragmites australis* through mowing or cutting and then convert the biomass to energy through combustion, biofuel production, or biogas production (Kobbing, Thevs, & Zerbe, 2013).

Of these three options, converting *Phragmites australis* to energy through biogas production by anaerobic digestion is particularly suited for the extensive agricultural industry and use of anaerobic digestion on farms in Wisconsin. Wisconsin currently has over 20 farm-based anaerobic digesters, which is the most in the U.S. (Agency, 2015). In addition, laboratory tests using *Phragmites australis* as a substrate in anaerobic digestion has been shown to increase methane production by 200 to 300 L of methane per kg of biomass and yield a methane content of between 55 and 60% of the total biogas volume (Köbbing et al., 2014). Although, it should be noted that Köbbing et al., 2014 did not indicate whether these values were based on bulk weight, dry weight, or dry volatile solids (VS) weight of *Phragmites australis* nor any detail regarding how the experiments were performed. In addition, Baute (2015), also observed an increase in methane gas of 115 L of methane per kg of *Phragmites australis* on a dry weight basis (140 L of methane per kg of *Phragmites australis* on a dry VS weight basis) using laboratory experiments on finely chopped (to less than 1 cm particles) *Phragmites australis*. The methane in the biogas can then be converted to heat or electrical energy via a boiler, engine, or turbine to offset other energy usage costs from harvesting (either directly or through a fee, depending on the harvester entity and digester owner agreement). If enough biogas can be produced from *Phragmites australis*, this control option could negate harvest costs or even generate a net profit. The resulting digestate can also be used as a fertilizer, which would further offset harvesting costs.

The biogas production potential of *Phragmites australis* is influenced by three main factors: 1) the season the plant is harvested, 2) the maturity of the plant when harvested, and 3) the extent the plant is homogenized prior to digestion (i.e., pretreatment). Previous research has focused on the first two factors and has found that harvesting green *Phragmites australis* in May to October, when the plant's nutrient content is the highest and its lignin content is lowest, is optimal for anaerobic digestion (Hansson & Fredriksson, 2004; Kask, 2011). In all of these studies, however, biogas and methane production were evaluated based on chopped *Phragmites australis*. It is expected that further pretreatment of the chopped *Phragmites australis*, which includes an increased breakdown of the plant's cellulose and lignin components resulting in increased exposure of the plant's lipids, proteins, and carbohydrates for conversion by microorganisms, will improve biogas and methane production.

Several physical, chemical, and biological pretreatment methods have

been evaluated on various crops and other substrates, including municipal wastewater sludge, and pig and cow manure for anaerobic digestion (Table 1). Most of the treatments in Table 1 show an increase in biogas production due to the pretreatment, with the exception of Thermal, which may result in a reduction in biogas production (-8%). In addition, no published data could be found regarding Ultrasound pretreatment, although it had been discussed as a potential option. However, there has been no research examining the impact of different pretreatment methods on biogas and methane production in anaerobic digestion of *Phragmites australis*. In addition, because the previous studies compared different substrates with different conditions (i.e., particle size, temperatures, incubation times, etc.) the results are difficult to compare and there is a need for research directly comparing pretreatment options for a single crop using a single method.

Table 1. Common anaerobic digestion pretreatment methods.

NDA – No data available.

Method	Description	Biogas Change (%)
Physical		
Mechanical—Cutting/Shredding	Cut substrate particles to 1 to 2 mm	10-25 (Menind, 2010)
Mechanical—Grinding	Grind substrate to particles less than 1 mm	60-80 (Menardo et al., 2012; Mshandete et al., 2006)
Thermal	Heat substrate to greater than 190° C under pressure for ≥ 1 hour to breakdown substrate	-8-25 (Liu et al., 2012) (Ma et al., 2011)
Ultrasound	Use of ultrasonic vibrations at over a 20 kHz frequency to breakdown substrate	NDA
Chemical		
Alkali	Addition of a base to breakdown substrate	20 (Liew et al., 2011)
Acid	Addition of a acid to breakdown substrate	31-74 (Chen et al., 2007)
Biological		
Aerobic	The use of aerobic, naturally occurring microorganisms breakdown substrate	30-40 (Muthangya et al., 2009)
Anaerobic (i.e., pre-acidification)	Use of anaerobic microorganisms to complete the first stage of anaerobic digestion (i.e., hydrolysis and acid production) and breakdown substrate	21 (Liu et al., 2012)
Enzymatic	Use of cellulose, hemicellulose, pectin, and starch-degrading enzymes to breakdown substrate	NDA

Thus, this study evaluated the impact of several pretreatment methods on the amount of biogas and methane that can be produced from anaerobically digesting *Phragmites australis* . The pretreatment methods that were evaluated, included 1) no pretreatment, 2) mechanical—cutting/shredding, 3) mechanical—grinding, 4) thermal, 5) ultrasound, 6) alkali, 7) acid, 8) aerobic, and 9) anaerobic.

Methods and Materials

Harvesting *Phragmites australis*.

Only the stalks of *Phragmites australis* were used for the analyses in this study, as it was assumed to make up the majority of the mass of the plant. Stalks were harvested from three different locations on the University of Wisconsin—Green Bay (UWGB) campus immediately prior to analytical and pretreatment analyses to avoid any potential changes that could have occurred if the *Phragmites australis* were stored. Green *Phragmites australis* was harvested between May and July of 2016, to optimize anaerobic digestion (Hansson & Fredriksson, 2004; Kask, 2011). For each harvest, five to 10 stalks with consistent maturity (e.g., color, diameter of stalk, and height) were randomly selected and cut using scissors. Once in the laboratory, the stalks were cut to 7.6 cm sections, mixed, and processed further using the methods outlined in the following sections.

Analytical Methods.

Chemical oxygen demand (COD), total solids (TS) and volatile solids (VS) were measured on 10 g of the harvested *Phragmites australis*. COD (mercuric digestion method) and nitrate (cadmium reduction method) were measured using HACH reagents (Loveland, CO). COD was only measured on *Phragmites australis* shredded with a Capresso coffee grinder (JURA, Inc., Niederbuchsiten, Switzerland), which represented the maximum potential COD, which was supported by the biogas and methane production results below. Briefly, 10 g of harvested *Phragmites australis* was shredded with 200 mL of DI water. Following shredding, 200 µL of the liquid fraction was used for COD analysis. TS and VS analyses were completed according to Section 2450 of the Standard Methods for the Examination of Water and Wastewater (American Public Health et al., 2005).

Biomethane Potential (BMP) Assay.

The 30-day biomethane potential (BMP) assay was used to evaluate the production of biogas and methane from each pretreatment option (Owen et al., 1979). The BMP assay was completed in Kimble (Neutraglas) 200 mL glass serum bottles (Kimble Chase Life Science and Research Products, LLC, Rockwood, TN) in triplicates for the control and each of the pretreatment options. Briefly, 50 mL of inoculum (Pagel's Ponderosa, LLC, Kewaunee, WI) was added to each of the 50 mL serum bottles. Inoculum was degassed for three days prior to use. Fresh inoculum was obtained prior to each BMP assay batch. 0.213 g of pretreated *Phragmites australis*, which corresponds to 125 g of COD, and 10 mL of de-ionized (DI) water were mixed in 50 mL tubes and added to the pretreatment replicate serum bottles. The DI water completely rinsed the pretreatment *Phragmites australis* into the serum bottle and captured any dissolved COD or loose plant material, which resulted from the pretreatment. To the control

serum bottles, 10 mL of DI water was added to maintain volume consistency with the pretreatment replicates. Chemglass Life Sciences 20 mm, blue, butyl rubber stoppers (Chemglass Life Sciences, Vineland, NJ) and Chemglass Life Sciences 20 mm open-hole, aluminum crimp cap seals (Chemglass Life Sciences, Vineland, NJ) were placed on each serum bottles and sealed using a Wheaton, E-Z Crimper (Wheaton Science Products, Inc., Millville, NJ). To establish anaerobic conditions, the headspace of the serum bottles was evacuated for three minutes using a Gast 115 volt, 4.2 amp, 60 hz pump (Gast Manufacturing, Inc., Benton Harbor, MI) and then replaced with a food grade mixture of compressed gas (30% CO2/70% N2) (Airgas, Radnor, PA) to 5 psi. The evacuation/replacement process was repeated three times. Following the third headspace evacuation and replacement, the excess gas was released by bubbling the gas into a beaker of water, allowing the replaced headspace to reach atmospheric pressure without introducing oxygen.

The serum bottles were then placed in an aluminum foil-covered Thermo Fisher Scientific MaxQ 4000 Benchtop Orbital Shaker/Incubator at 125 rpm and 35°C (Thermo Fisher Scientific, Waltham, MA). The serum bottles remained in the shaker/incubator for 30 days. On Days 1, 3, 7, 14, 21, and 30, the serum bottles were removed from the shaker/incubator and biogas volume was measured using a wetted 100 mL Fortuna Air-Tite glass syringe (Air-Tite Products Co., Inc., Virginia Beach, VA) with a 22 gauge BD needle (Becton, Dickinson, and Company, Franklin Lakes, NJ). Biogas was returned to the serum bottle after volume measurement. On Day 30, methane content was measured using a GOW-MAC Series 300 gas chromatographer with a thermal conductivity detector (GC-TCD) (Bridgewater, NJ). A Grace Alltech 4 ft by ¼ in 20% Carb 20M on chrom 80/100 mesh PoraPak column and a Grace Alltech 6 ft by ¼ in Q 80/100 PoraPak column were used for the analysis (Grace Discovery Sciences, Columbia, MD). Briefly, on Day 30, 50 µL of sample was removed from each serum bottle using a 100 µL Hamilton gas-tight, glass syringe (Reno, Nevada) and injected into the GC-TCD. A five-point standard curve (0, 20, 50, 70, and 100% methane) was established for the GC-TCD results. Chemically pure methane was used for the standard curve (Airgas, Radnor, PA). The standard curve had a coefficient of determination (R2) of 0.9996 and was repeated for each BMP assay batch.

Pretreatment Methods.

The pretreatment methods that were evaluated included 1) no pretreatment, 2) mechanical—cutting/shredding, 3) mechanical—grinding, 4) thermal, 5) ultrasound, 6) alkali, 7) acid, 8) aerobic, and 9) anaerobic. Each pretreatment was completed in triplicate on 0.213 g of harvested *Phragmites australis*, which corresponded to 125 mg of COD. Each time a set of pretreatments were completed and analyzed using the BMP assay, triplicate control BMP assays were also completed. As stated previously, the control BMP assay replicates received only inoculum and no *Phragmites australis*. Thus, each set of controls served as the no pretreatment. In addition, each of the pretreatment methods was completed in the

laboratory using methods simulating full-size versions of the technology.

Mechanical—cutting/shredding: Samples were cut/shredded using a Capresso coffee grinder (JURA, Inc., Niederbuchsiten, Switzerland). The mechanically cut/shredded *Phragmites australis* was then placed in 50 mL glass test tubes with 10 mL of DI water.

Mechanical—grinding: Samples were ground with a glass mortar and pestle and rinsed off of the mortar and pestle using 10 mL of DI water. The mechanically ground *Phragmites australis* and DI water were then poured into 50 mL glass test tubes.

Thermal: Samples were heated to 190°C for 1 hour in 50 mL glass test tubes using a Precision Scientific Thelco Laboratory oven (Precision Scientific Co., Teynampet, Chennai, India). Following the pretreatment, 10 mL of DI water was added to the test tubes.

Ultrasound: Samples were placed in 50 mL glass test tubes and 10 mL of DI water. The test tubes were then sonicated at 20 kHz for 4 hours using a Branson 2200 sonicator (Branson, Inc., Danburk, CT).

Alkali: Samples were soaked in a bath of dilute (2%) sodium hydroxide (Sigma-Aldrich, Milaukee, WI) for 60 min and at 120°C. Following pretreatment, samples were strained using 0.5 mm diameter stainless steel screen mesh (Menards, Green Bay, WI) and placed in 50 mL test tubes with 10 mL of DI.

Acid: Samples were soaked in a bath of dilute (1%) hydrochloric acid (Sigma-Aldrich, Milwaukee, WI) for 60 min and at 120°C, strained using stainless steel screen mesh (Menards, Green Bay, WI), and placed in 50 mL test tubes with 10 mL of DI.

Aerobic: Samples were added to aerated, activated sludge obtained from the Green Bay Wastewater Treatment Plant (aka, NEW Water, Green Bay, WI). Aeration was completed using a Topfin Air 8000 fish tank pump (PetSmart, Phoenix, AZ) and diffuser to obtain a dissolved oxygen level of 2 mg/L. Samples were then strained using stainless steel screen mesh, rinsed with DI water, and placed in 50 mL test tubes with 10 mL of DI water.

Anaerobic: Samples were placed in 50 mL glass test tubes with 10 mL of DI water and then added to the 200 mL serum bottles, which contained 25 mL of inoculum (described previously). Chemglass Life Sciences 20 mm, blue, butyl rubber stoppers (Chemglass Life Sciences, Vineland, NJ) and Chemglass Life Sciences 20 mm open-hole, aluminum crimp cap seals (Chemglass Life Sciences, Vineland, NJ) were placed on each serum bottles and sealed using a Wheaton, E-Z Crimper (Wheaton Science Products, Inc., Millville, NJ). The headspace of the serum bottles was then evacuated and replaced with 30% CO2/70% N2 gas to establish anaerobic conditions. The serum bottles were then placed on the shaker/incubator at 150 rpm and 35°C for 4 hours. Following 4 hours, the serum bottles were opened and 25 mL of additional inoculum was added to each. The BMP assay procedure was then carried out as described previously.

Statistical Analysis.

Experimental values are reported as the mean ± standard error.

Pretreatments and controls were compared using, the unpaired, two-tailed Student's t-test in Excel. Differences were considered significant for p-values ≤ 0.05 and marginal significance was considered for p-values ≤ 0.10.

Results and Discussion

Analytical.

The COD of the shredded Phragmites australis was 586 ± 77.3 mg COD/g Phragmites australis; TS and VS were 54.6 ± 0.88% and 36.2 ± 1.71%, respectively.

Biogas Production.

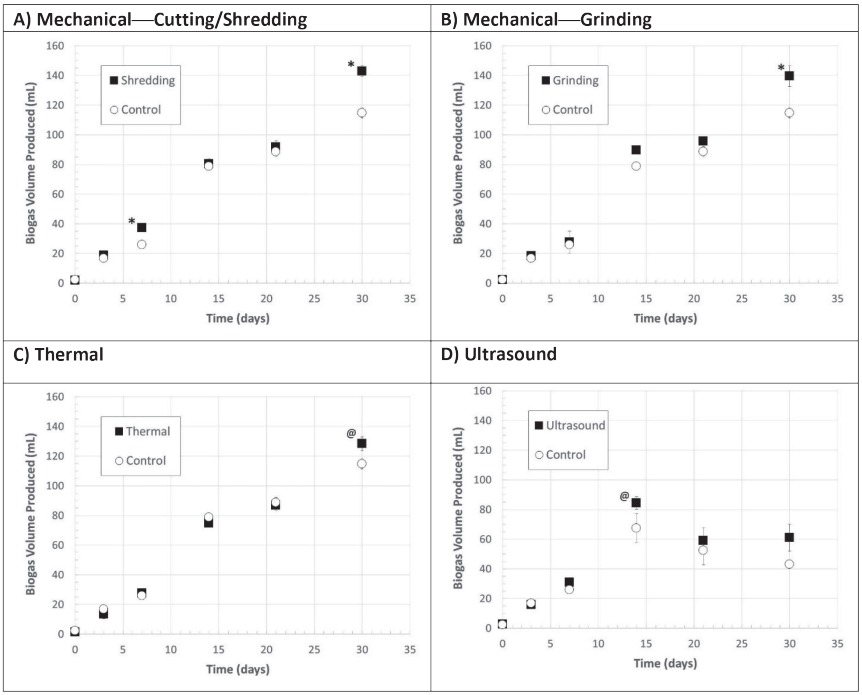
The pretreatments were run in four batches, each with separate controls, due to the limited number of serum bottles and spaces in the shaker/incubator. Batch 1 consisted of mechanical—cutting/shredding, mechanical—grinding, and thermal pretreatments. Ultrasound pretreatment was completed alone for Batch 2.l Batch 3 and 4 consisted of alkali and acid and aerobic and anaerobic, respectively.

Mechanical—cutting/shredding and mechanical—grinding pretreatments ($p \leq 0.05$) resulted in increased biogas production. At the end of pretreatment (30 days), mechanical—shredding resulted in 24.7% greater gas production than the control, which was similar to the 10-25% observed previously (Figure 1) (Menind, 2010). Mechanical—grinding pretreatment resulted in 21.7% greater gas production than the control, but was much lower than the 60-80% observed previously (Figure 1) (Menardo et al., 2012; Mshandete et al., 2006). These results were expected, as the previous studies dried the biomass before grinding with a laboratory mill, which resulted in much smaller (2 mm) and more discrete particles, increased breakdown of the cellulose and lignin, and greater gas production. Thermal pretreatment produced 11.9% more biogas ($p \leq 0.10$) within the range (-8-25) observed previously (Figure 1) (Liu et al., 2012; Ma et al., 2011). However, the BMP assays for mechanical—cutting/shredding, mechanical—grinding, and thermal pretreatments, as well as, the respective controls should have been run for a longer period of time. Typically, a BMP assay is set up so that the quantity of biomass and/or inoculum added results in maximum biogas production prior to Day 30, which was not observed for these pretreatments. The biogas production measured on Day 30 for the mechanical—cutting/shredding, mechanical—grinding, and thermal pretreatments may not have been at the maximum. In addition, whether or not the control and pretreatments continue to be significantly different until maximum gas production is reached, could not be determined. Thus, additional tests should be completed to verify these results.

Ultrasound pretreatment was only marginally significantly greater than the control on Day 15, which was also when maximum biogas production was observed. These results indicate that the BMP assay for ultrasound was run for a sufficient length of time, as maximum biogas production was reached prior to Day 30. These results also show that ultrasound pretreatment may produce more biogas production in the short-term (less than 15 days), but not over the long term

(greater than 15 days). By Day 30, there was no difference in biogas production between the pretreatment and the control. The decrease in both the pretreatment and the control after Day 15 indicates that there was some biogas loss, which could have been due to septa leaks or leaks in the syringe during volume measurement. It was assumed that the biogas loss was consistent between the pretreatment and the controls. However, additional tests should be completed to verify the results presented herein.

Figure 1. Mechanical—Cutting/Shredding, Mechanical—Grinding, and Thermal retreatment biogas production. (*) indicates statistical significance from the control (p-value ≤ 0.05). (@) indicates marginal statistical significance from the control (p-value ≤0.10).

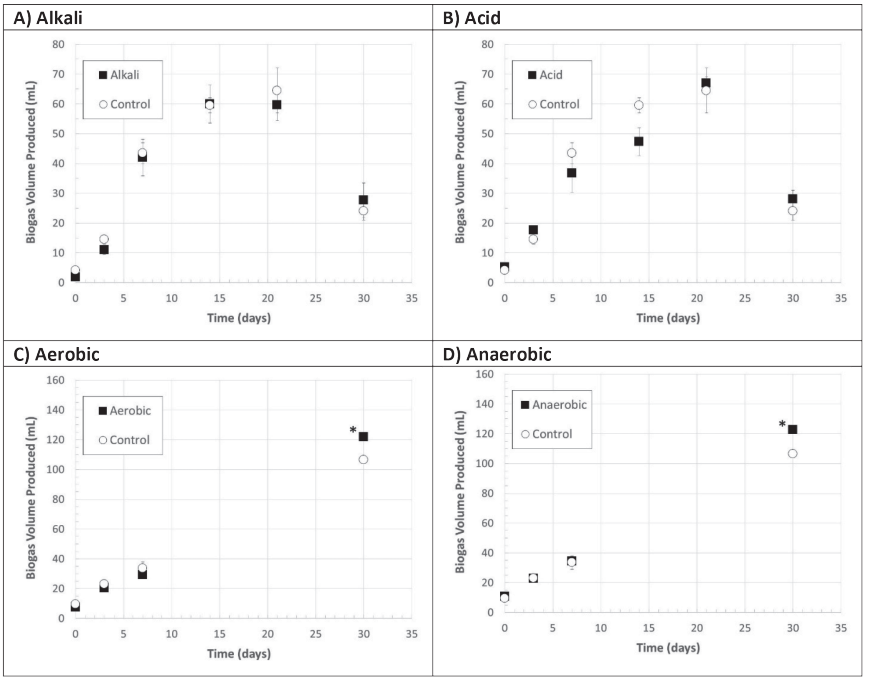


There was no significant difference between the alkali and acid pretreatment biogas production and the control biogas production (Figure 2). Previous studies observed a 20% increase due to alkali and a 31-74% increase due to acid pretreatment (Chen et al., 2007; Liew et al., 2011). For both the alkali and acid pretreatment and the controls, biogas production decreased after Day 21, again likely due to leaks in the septa or leaks in the syringe during volume measurement. It was assumed that the biogas loss was consistent between the pretreatment and the controls, but additional tests should be completed to verify the results.

Both aerobic and anaerobic pretreatment biogas production was

significantly greater than the control after 30 days (Figure 2) ($p \leq 0.05$). Aerobic pretreatment resulted in 14.6% more biogas than the control, which is lower than the 30-40% observed previously (Muthangya et al., 2009). Anaerobic pretreatment resulted in 15.2% more biogas than the control, which was also lower than the 21% observed previously (Liu et al., 2012). Day 14 and 21 results are missing because the syringe was damaged. In addition, similar to the mechanical—cutting/shredding, mechanical—grinding, and thermal pretreatments, the aerobic and anaerobic pretreatment BMP assays should have been run longer or the quantity of biomass and inoculum should have been changed so that maximum biogas production could have been observed. Additional tests should be completed to verify these results.

Figure 2. Alkali, acid, aerobic, and anaerobic pretreatment biogas production. (*) indicates



statistical significance from the control (p-value ≤ 0.05). (@) indicates marginal statistical significance from the control (p-value ≤0.10).

Methane Content.

Methane content was measured on Day 30 using the GC-TCD. There was no significant difference between the methane content of any of the pretreatments and the respective controls (Figure 3). These results indicate that while several *Phragmites australis* pretreatment methods resulted in greater biogas production, both aerobic and anaerobic pretreatment biogas production was significantly greater than the control after 30 days (Figure 2) ($p \leq 0.05$). Aerobic pretreatment

resulted in 14.6% more biogas than the control, which is lower than the 30-40% observed previously (Muthangya et al., 2009). Anaerobic pretreatment resulted in 15.2% more biogas than the control, which was also lower than the 21% observed previously (Liu et al., 2012). Day 14 and 21 results are missing because the syringe was damaged. In addition, similar to the mechanical—cutting/shredding, mechanical—grinding, and thermal pretreatments, the aerobic and anaerobic pretreatment BMP assays should have been run longer or the quantity of biomass and inoculum should have been changed so that maximum biogas production could have been observed. Additional tests should be completed to verify these results.

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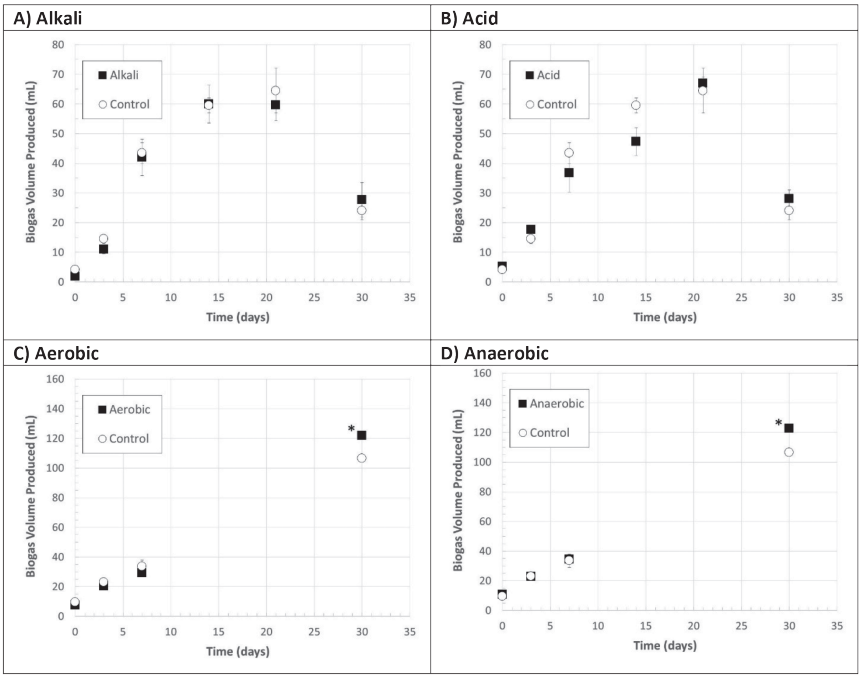


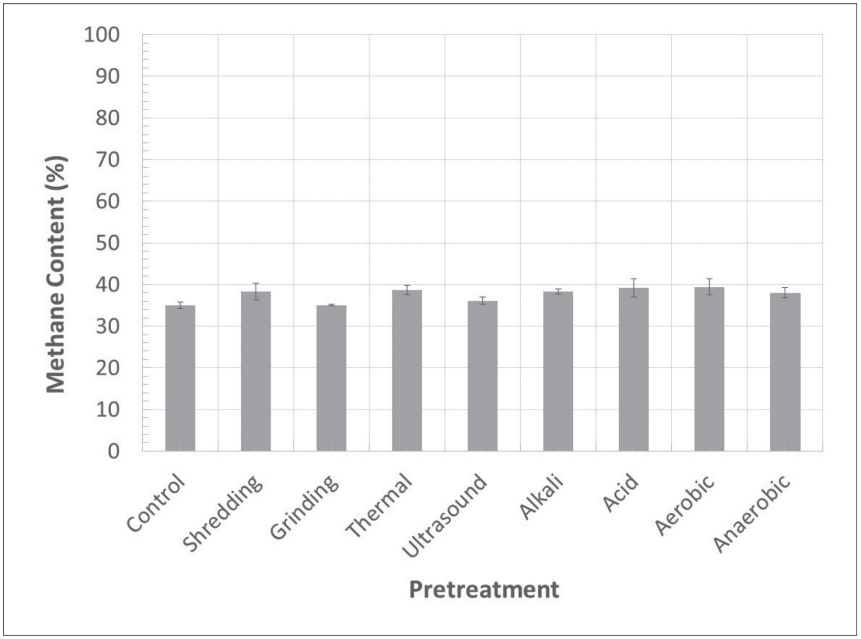
statistical significance from the control (p-value ≤ 0.05). (@) indicates marginal statistical significance from the control (p-value ≤0.10).

Methane Content.

Methane content was measured on Day 30 using the GC-TCD. There was no significant difference between the methane content of any of the pretreatments and the respective controls (Figure 3). These results indicate that while several *Phragmites australis* pretreatment methods resulted in greater biogas production (i.e., mechanical—cutting/shredding, mechanical—grinding, thermal, aerobic, and anaerobic pretreatments after 30 days and alkali and acid pretreatments on Day 15), the pretreatment did not change the percentage of methane in the biogas. The average methane content of the pretreatments was 37.9 ± 0.52%, which was

much lower than the 55-60% observed previously (Köbbing et al., 2014).

Figure 3. Pretreatment methane content results. (*) indicates statistical significance from the control



(p-value ≤ 0.05). (@) indicates marginal statistical significance from the control (p-value ≤0.10).

Methane Production.

Typically, as in the case of the mechanical—cutting/shreding, mechanical—grinding, thermal, aerobic and anaerobic pretreatments, maximum biogas volume production occurred on Day 30. For the ultrasound pretreatment, maximum biogas production occurred on Day 14. For the alkali pretreatment, maximum biogas production occurred on Day 14. However, the biogas production with the alkali treatment on Day 14 was only 0.3 mL lower than that on Day 21, and because the corresponding control for the alkali pretreatment reached the maximum biogas production on Day 21, the Day 21 values were used in the calculation of methane production. For the acid pretreatment, maximum biogas production occurred on Day 21. Because methane content was measured on Day 30 and maximum biogas production for the ultrasound, alkali, and acid pretreatments occurred prior to Day 30, it was assumed that methane content did not significantly change over the extent of the experiment. This assumption is consistent with the methane content results (Figure 3), which show that exposure of the *Phragmites australis* to the inoculum resulted in no difference in the percentage of methane in the biogas compared to when no *Phragmites australis* was present (i.e., the control).

To compare the average additional methane production for each pretreatment, the average methane production of the control was subtracted from average methane production of the corresponding pretreatments (Figure 4). Importantly, however, statistical significance was calculated on the average pretreatment methane production values and the average methane production values of the corresponding control (i.e., prior to subtraction). Mechanical—cutting/shredding, mechanical—grinding, thermal, aerobic, and anaerobic pretreatments increased methane production ($p \leq 0.05$). Mechanical—cutting/shredding, mechanical—grinding and thermal pretreatments had the most effect with production increased between 40 to nearly 70%.

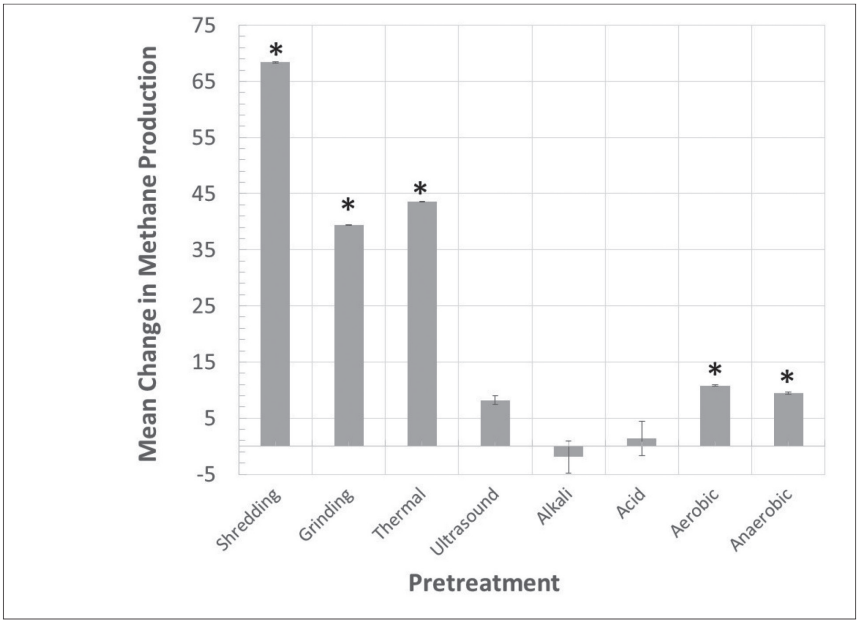


Figure 4. Average change in methane production of pretreatment. (*) indicates statistical significance from the control (p-value ≤ 0.05). (@) indicates marginal statistical significance from the control (p-value ≤0.10).

Converting our laboratory values to production level values on a dry TS and VS weight (Table 1) illustrates that additional methane production due to mechanical—cutting/shredding, mechanical—grinding, thermal, aerobic, and anaerobic pretreatment is comparable to the 200 to 300 L of methane produced per kg of *Phragmites australis* observed previously (Köbbing et al., 2014). Again, it should be noted that Köbbing et al., (2014) did not indicate whether these values were based on bulk weight, dry weight, or dry volatile solids (VS) weight of *Phragmites australis*. The results are also comparable to the 114 L of methane per kg of *Phragmites australis* on a dry weight basis (140 L of methane per kg of *Phragmites australis* on a dry VS weight basis) reported by Baute (2015) for

finely chopped *Phragmites australis*.

Table 2. Additional methane production with mechanical—cutting/shredding, mechanical—grinding, thermal, aerobic, and anaerobic pretreatment compared to no pretreatment.

Pretreatment	Additional Methane Produced (L/kg, Dry TS Weight)	Additional Methane Production (L/kg, Dry VS Weight)
Shredding	125	346
Grinding	72.3	200
Thermal	79.8	220
Aerobic	19.8	54.8
Anaerobic	17.4	48.0

Conclusions

Overall, the results of this study show that mechanical—cutting/shredding, mechanical—grinding, thermal, ultrasound aerobic, and anaerobic pretreatments resulted in a significant increase in overall biogas production. Mechanical shredding likely resulted in the greatest increase in biogas and methane production because of increased breakdown of the cellulose and lignin components of the *Phragmites australis*. While no pretreatment affected the methane content in the biogas, shredding, grinding, thermal, aerobic, and anaerobic pretreatments resulted in a significant increase in methane production. Thus, utilizing these pretreatment methods in combination with anaerobic digestion in an overall *Phragmites australis* mitigation strategy could result in some cost-offsetting. Additional research is needed to determine the potential cost savings of these pretreatment methods in a working agricultural setting. Additional factors to investigate would include the quantity of *Phragmites australis* available for harvest in Wisconsin and the costs associated with harvesting and transporting the *Phragmites australis* to anaerobic digester. In addition, future research should repeat the experiments presented herein with inoculum that is mixed and degassed over a longer period of time, so maximum biogas production can be reached prior to 30 days.

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