**Comparison of liquid hot water and alkaline pretreatments of giant reed for improved
enzymatic digestibility and biogas energy production**

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Abstract:

Liquid hot water (LHW) and alkaline pretreatments of giant reed biomass were compared in terms of digestibility, methane production, and cost-benefit efficiency for electricity generation via anaerobic digestion with a combined heat and power system. Compared to LHW pretreatment, alkaline pretreatment retained more of the dry matter in giant reed biomass solids due to less severe conditions. Under their optimal conditions, LHW pretreatment (190 °C, 15 min) and alkaline pretreatment (20 g/L of NaOH, 24h) improved glucose yield from giant reed by more than 2-fold, while only the alkaline pretreatment significantly ($p < 0.05$) increased cumulative methane yield (by 63%) over that of untreated biomass (217 L/kg VS). LHW pretreatment obtained negative net electrical energy production due to high energy input. Alkaline pretreatment achieved 27% higher net electrical energy production than that of non-pretreatment (3,859 kJ/kg initial total solids), but alkaline liquor reuse is needed for improved net benefit.

Keywords: Giant reed; Liquid hot water; Alkaline pretreatment; Methane yield, Benefit-cost analysis

1. Introduction

Arundo donax L. (giant reed) is a perennial rhizomatous grass native to Asia, and currently grows in various regions in many countries, including Italy, Portugal, Spain, France, Greece, China, Australia, the United States, and Brazil (e Silva et al., 2015; Ge et al., 2016). Giant reed is a promising crop for bioenergy production, due to its attractive features including high biomass yield, ability to grow in different types of soils and weather conditions, lower tillage requirements, and phytoremediation properties compared to traditional crops (Ge et al., 2016). Methane production from giant reed via anaerobic digestion has recently been suggested because of the simple process, reliable performance, and low greenhouse gas emissions. For example, since 2013 giant reed has been grown on 25 farms in Italy for methane production (Corno et al., 2015). Giant reed is a lignocellulosic biomass with a complex structure formed by cellulose, hemicellulose and lignin, which makes it considerably recalcitrant to digestion. In order to enhance the digestibility of giant reed and/or its subsequent methane yield by anaerobic digestion (AD), pretreatment has been studied using different methods, such as diluted acid, steam explosion, cellulose solvent-based lignocellulosic fractionation (CSLF), liquid hot water (LHW), and alkaline (Di Girolamo et al., 2014; Ge et al., 2016).

Liquid hot water (LHW) is one of the pretreatment methods that increase the accessibility of cellulose in the network of lignocellulose by solubilizing hemicellulose using elevated temperature and pressure (Di Girolamo et al., 2014). Organic acids, such as acetic acid, generated from the LHW process can further catalyze the hydrolysis of hemicellulose into mono-sugars (Hendriks and Zeeman, 2009; Wan et al., 2011). According to Di Girolamo et al. (2013), LHW pretreatment of giant reed at 180 °C for 10 min caused a 23% increase in methane yield. Compared to other leading pretreatments (e.g., diluted acid, lime, soaking in ammonia, ammonia

fiber explosion (AFEX), sulfur dioxide-impregnated steam explosion), the major advantages of LHW are that it is chemical free and does not require corrosion-resistant materials for reactors (Bacovsky et al., 2013; Taherzadeh and Karimi, 2008; Zhuang et al., 2016). In addition, LHW generates fewer inhibitory compounds than steam explosion, which also uses high temperature and pressure for pretreatment (Zhuang et al., 2016).

Alkaline pretreatment is one of the leading chemical pretreatment methods (Bali et al., 2015; Gu et al., 2015; Liang et al., 2014). Alkalis can cleave lignin-carbohydrate linkages, dissolve lignin, or alter the structure of lignin in lignocellulosic biomass (Yang et al., 2015). Since lignin is the most recalcitrant component of lignocellulose, alkaline pretreatment is generally effective in improving digestibility of lignocellulosic biomass, especially agricultural residues and herbaceous crops (Galbe and Zacchi, 2007). In one study that used a mild alkali (6 g/L NaOH) to pretreat different biomass crops and residues, giant reed showed a 30% increase in cumulative methane yield (Di Girolamo et al., 2014). Alkaline pretreatment can be conducted at room temperature without a complicated reactor, which makes it highly attractive for on-farm application (Wan et al., 2011). A common problem of alkaline or other chemical pretreatment methods is that the pretreated biomass needs to be washed or neutralized prior to enzymatic hydrolysis or the AD process, and thus consumes more chemicals and/or generates additional wastewater (Wan et al., 2011). In addition, remaining alkali in the pretreatment liquor is usually reused, in order to reduce the environmental impacts of chemical waste disposal and costs for chemicals (Wan et al., 2011).

Currently, research on pretreatment of giant reed is still limited compared to other energy crops, such as miscanthus. According to previous studies, the biomass composition and structure of giant reed may vary with different cultivation conditions and harvest time, which would

further affect its digestibility and the performance of the subsequent methane production by AD (Liu et al., 2016). To date, there have been no reports on comparison of different pretreatment methods, such as LHW and alkaline, using giant reed harvested at the same time and location (Ge et al., 2016). As a result, it is still unclear which pretreatment is more effective for giant reed. Besides, pretreatment has common issues, such as the loss of biomass and requirement of additional energy and/or chemical inputs, which may offset the improved energy production in terms of net benefit. To the best of the authors' knowledge, there have been no reports that evaluated and compared the performance between LHW and alkaline pretreatment of giant reed, considering energy/chemical inputs and energy outputs.

The objective of this study was to fill these research gaps by conducting a side-by-side comparison of LHW and alkaline pretreatment for methane production from giant reed. Using the same source of giant reed biomass, LHW pretreatment was conducted at different temperatures for different times, and alkaline pretreatment with different loadings was conducted. The effects of LHW and alkaline pretreatments on biomass recovery, sugar yield by enzymatic hydrolysis, and methane yield by AD were evaluated and compared. Net biogas energy production and cost efficiency for methane production from giant reed with these two pretreatment methods were also estimated.

2. Methods

2.1. Raw materials and inoculum

Giant reed, collected from The Ohio State University (OSU) research farm (Columbus, OH, USA) on December 10, 2014, was ground to pass a ½ inch sieve using a shredder-chipper (Mighty Mac, Mackissic Inc., Parker Ford, PA, USA), and stored in an airtight container at room

temperature prior to use. Effluent from a mesophilic liquid anaerobic digester (KB BioEnergy, Akron, OH, USA) that was fed with sewage was used as an inoculum for AD. The effluent was stored at 4 °C after collection, and acclimated at 37 °C for 1 week before use. Characteristics of the untreated giant reed biomass and inoculum for AD are presented in Table 1.

2.2. LHW pretreatment

LHW pretreatment was conducted in a 1-L Parr reactor (Parr Instrument Company, Moline, IL, USA) according to Wan and Li (2011). The Parr reactor was filled with 20 g of giant reed (dry mass) and 200 mL of deionized (DI) water to reach a solid to liquid ratio of 1:10 (w/v). The Parr reactor was heated with a heating mantle to a target temperature (170 °C, 190 °C, 210 °C, or 230 °C) in 30-45min, held at the target temperature for a designated period of time (5, 10, or 15 min) and cooled down to room temperature by cooling water in about 30 min. During the whole pretreatment process, the Parr reactor was agitated continuously at approximately 400 rpm. The pretreated slurry was washed with 1000 mL of DI water and filtered by vacuum filtration using Whatman #1 filter paper to separate the water-insoluble residues and liquor. Then, the water-insoluble residues and liquor were collected separately for further analysis (Wan et al., 2011). A severity factor equation was used to characterize the combined effects of pretreatment temperature (T) and time (t) (Kim et al., 2014; Overend et al., 1987). The equation is as follows:

$$\log R_0 \ (R_0 = t \times \exp ((T - 100)/\omega)) \quad (1)$$

where t is pretreatment time, T is pretreatment temperature, $\omega = 4.6$ is an empirical parameter.

2.3. Alkaline pretreatment

Twenty grams of giant reed (dry mass) were added to 200 mL of NaOH solution in a 500-mL flask with NaOH concentration of 1, 3, 5, 7, 12, or 20 (g/L). The loading ratios were 1%, 3%, 5%, 7%, 12%, and 20% (g NaOH/g initial TS of giant reed biomass), respectively. The flasks were covered with Parafilm on the top and incubated at room temperature (24±1 °C). After 24 h, the pretreated giant reed was washed with tap water using a sieve (325 Mesh) until the pH reached around 8, and then further neutralized with 1 M HCl to reach a pH value of about 7. The neutralized material was then drained, washed, and dried for further analysis.

2.4. Enzymatic hydrolysis

In order to determine enzymatic digestibility of giant reed biomass, enzymatic hydrolysis of untreated and pretreated giant reed was conducted based on a Laboratory Analytical Procedure reported by the National Renewable Energy Laboratory (NREL) (Selig et al., 2008). Briefly, about 0.25 g giant reed (dry mass) was mixed with 10 mL of 0.05 M citrate buffer (pH 4.8) in a 40-mL tube. The Cellic CTec 2 (Novozymes, Bagsværd, Denmark) was loaded into the mixture at 20 FPU/g solid. The tube was then incubated at 50 °C with shaking at 200 rpm for 72 h. After that, the hydrolysate was filtered using Whatman #1 filter paper, and the filtrate was collected in a 20-mL tube which was then boiled for 5 min to deactivate enzymes. The deactivated filtrate was further centrifuged at 7826 × g for 10 min. The centrifuged supernatant was filtered through a 0.2 µm nylon filter before analysis by HPLC.

The glucose yield and enzymatic digestibility were calculated using the following equations:

$$Glucose\ yield\ (\%) = \frac{M_{glucose}}{f \times M_{cellulose}} \times 100 \quad (2)$$

$$\text{Enzymatic digestibility (\%)} = \frac{M_{\text{glucose}}}{f \times N_{\text{cellulose}}} \times 100 \quad (3)$$

where M_{glucose} is the amount of glucose released from pretreated giant reed by enzymatic hydrolysis; $M_{\text{cellulose}}$ is the amount of cellulose in raw giant reed before pretreatment; $N_{\text{cellulose}}$ is the amount of cellulose in pretreated giant reed; and $f = 1.11$ is the conversion factor for cellulose to glucose (Liu et al., 2016).

2.5. Anaerobic digestion of giant reed

Giant reed biomass (feedstock) was mixed with AD effluent (inoculum) to obtain a feedstock to inoculum (F/I) ratio of 0.5 (w/w, based on volatile solids (VS)), and DI water was added to adjust the total solids (TS) content to 5% (w/w). About 600 g of the mixture was loaded into a 1-L glass reactor which was sealed by a stopper with an outlet connected to a 5-L Tedlar gas bag (CEL Scientific, Santa Fe Springs, CA, USA) for biogas collection. The reactor was incubated at 37 ± 1 °C for 30 days. Biogas volume and composition were measured every 3-5 days. AD with only inoculum added was run as a control. Duplicate reactors were run for each pretreatment condition.

2.6. Analysis methods

The TS, VS, and pH of samples were measured following the Standard Methods for Examination of Water and Wastewater (APHA, 2005). An elemental analyzer (Elementar Vario Max CNS, Elementar Americas, Mt. Laurel, NJ, USA) was used to determine total carbon (TC) and total nitrogen (TN). The carbon to nitrogen (C/N) ratio was then determined based on the TC and TN contents. The total ammonia nitrogen (TAN) was determined by a modified distillation method (ISO, 1984) with a Kjeldahl Distillation Unit B-324 (Buchi, Labortechnik, AG,

Switzerland). Crude protein was calculated by determining total organic nitrogen (TN minus TAN) and then multiplying by a factor 6.25 (Hattingh et al., 1967). Dry matter recovery in the solids or liquor was calculated as a percentage of water-insoluble mass or water-soluble mass in the pretreated slurry to the TS in the untreated giant reed. Total dry matter recovery was defined as the sum of dry matter recovery in the solids and liquor.

Structural carbohydrates (cellulose, hemicellulose, and lignin) contents were measured following an NREL Laboratory Analytical Procedure (Sluiter et al., 2008). Briefly, samples were extracted with DI water and ethanol using Accelerated Solvent Extraction (ASE 350, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and then the extractive content was determined. The extractive-free samples were subjected to a two-stage acid hydrolysis to hydrolyze cellulose and hemicellulose into mono-sugars. A high performance liquid chromatography (HPLC) (LC-20AB, Shimadzu, Kyoto, Japan) equipped with an Aminex HPX-87P column (Bio-Rad, Inc., Hercules, CA, USA) and a refractive index detector (RID) was used to analyze sugars in the acid hydrolysate and enzymatic hydrolysate. The temperature of the column and detector were 60 and 55 °C, respectively. The mobile phase was HPLC grade water, eluting at a flow rate of 0.3 mL/min. Cellulose and hemicellulose contents were calculated based on the amounts of corresponding mono-sugars. The acid-insoluble lignin and acid-soluble lignin were determined by gravimetric analysis, and ultraviolet-visible (UV-vis) spectroscopy (BioMate 3S spectrophotometer, Waltham, MA, USA), respectively. Organic acids, HMF, and furfural were analyzed by HPLC with a Phenomex Rezex RFQ Acid column (Phenomex, Torrance, CA, USA). The mobile phase was 0.01 N sulfuric acid at a flow rate of 0.4 mL/min. The temperature of the column and detector were maintained at 45 and 55 °C, respectively.

The volume of biogas in the Tedlar gas bag was determined by a drum-type gas meter (Ritter, Bochum, Germany) and the composition of biogas (CO₂, CH₄, N₂, and O₂) was analyzed by a gas chromatograph (Agilent, HP 6890, Wilmington, DE, USA) equipped with a 30 m × 0.53 mm × 10 μm alumina/KCl deactivation column and a thermal conductivity detector (TCD). Helium gas was used as a carrier gas, and the flow rate was controlled at 5.2 mL/min. The column oven was initially set at 40 °C for 4 min and then increased to 60 °C with an increment of 20 °C /min and held at 60 °C for 5 min. The temperature of the column and detector were set at 40 °C and 200 °C, respectively. The CH₄ production contributed by inoculum (control) was subtracted from the measured value of each treatment.

2.7. Energy and cost analysis

Methane yield based on initial TS of giant reed biomass was calculated using Eq. (4):

$$CH_4 \text{ yield (L/kg initial TS)} = CH_4 \text{ yield (L/kg VS)} \times VS \times \text{Dry matter recovery} \quad (4)$$

Biogas energy was calculated by multiplying the initial TS-based methane yield by the lower heating value of methane (33 kJ/L at 25 °C and 101 kPa) and an assumed effective electric efficiency of 0.6 for a combined heat and power (CHP) system (USEPA, 2013). Heating for the AD digester was not calculated, since thermal energy produced by the CHP system can typically meet the heat load of a mesophilic digester according to an analysis reported by the U.S. EPA (USEPA and CHPP, 2007). However, additional energy is needed for heating giant reed biomass during LWH pretreatment, and this energy input was calculated using Eq. (5):

$$\text{Energy input for material heating (kJ/kg initial TS)} = M_W \times C_W \times \Delta T \quad (5)$$

where M_W is the liquid to solids ratio (200 kg/kg initial TS), C_W is the specific heat of water (4.2 kJ/ kg/ °C), and ΔT is the temperature increase (°C, from 25°C to the target temperature).

Net biogas energy (kJ/kg initial TS) was then calculated as biogas energy minus the energy input for material heating. The benefit of electricity generation (\$/tonne initial TS) was calculated by multiplying net biogas energy by 1000 (kg/tonne) and the average electricity price (\$ 2.89×10^{-5} /kJ or 10.42 cents/kWh) in the U.S. (USEIA, 2016). The cost of giant reed biomass production was assumed to be \$44/tonne according to the expected farm gate price (Ge et al., 2016). The cost for NaOH (\$/tonne initial TS) was estimated based on the NaOH loading ratios and the price of NaOH (\$450/tonne) (Kumar and Murthy, 2011). The net benefit for electricity generation from giant reed via AD with CHP was determined by subtracting the cost of giant reed biomass production and NaOH inputs from the benefit of electricity generation.

2.8. Statistical analysis

Statistical significance was assessed by analysis of variance (ANOVA) ($\alpha = 0.05$) using Minitab (Version 16, Minitab, Inc., State College, PA, USA).

3. Results and Discussion

3.1. Effect of pretreatment on mass recovery and composition of biomass

After LHW pretreatment with different temperatures (170-230 °C) and times (5-10 min), 55.2-84.5% of the dry matter was recovered in solids, and the recovery efficiency decreased substantially with the increase of severity (Table 2). About 14-25% of dry matter was dissolved into the liquor during LHW pretreatment. When the severity was increased from 7.3 to 9.7, the percentage of dry matter dissolved in the liquor increased, which was consistent with the decreased efficiency of dry matter recovery in the solids (Table 2). However, when the severity was further increased from 9.7 to 13.5, the percentage of dry matter dissolved in the liquor

decreased from 25.3% to 18.4%, with total recovery efficiencies as low as 73.5% (Table 2). This might be due to the formation of gaseous compounds under conditions of high severity as suggested by Turn et al. (1998) and Wan et al. (2011). In contrast, about 75-87% of dry matter was recovered in giant reed solids after alkaline pretreatment with 1-20 g/L of NaOH for 24 h, and higher alkaline loadings decreased dry matter recovery efficiency (Table 2). Alkaline pretreatment was conducted at room temperature, which was much less severe than the LHW pretreatment. As a result, alkaline pretreatment recovered more dry matter in giant reed solids than LHW pretreatment.

Both LHW pretreatment and alkaline pretreatment recovered more than 81% of cellulose in giant reed biomass, except for LHW pretreatment at 230 °C for 15 min, which recovered 72% (Table 2). Besides, both LHW pretreatment and alkaline pretreatment were effective in removal of lignin from giant reed solids, with lignin removal efficiencies of 22-54% and 32-55%, respectively (Table 2). However, LHW pretreatment and alkaline pretreatment showed a substantial difference in xylan recovery. Generally, xylan recovery efficiency after LHW pretreatment decreased from 83.7% to 0% with an increase of severity from 7.3 to 13.5 (Table 2). In contrast, xylan recovery efficiency after alkaline pretreatment stayed at relatively high levels (66.9-81.3%) with different NaOH loadings (Table 2).

Degradation products are generally formed from lignocellulose during pretreatment under severe conditions, such as high temperature. As shown in Figure 1, when the severity was in the range of 7.3-9.5, about 1.1 g of degradation products were found in the liquor, including propionic acid (0.21-0.22 g), acetic acid (0.38-0.70 g), and levulinic acid (0.09-0.17 g). Higher severity (9.7-11.6) resulted in greater amounts and more types of degradation products. Xylose, furfural and formic acid were also detected after LHW pretreatment with severities of 9.7-11.6

(Figure 1). All these degradation products, except levulinic acid, can be formed directly or indirectly from xylan under high temperature (Jönsson and Martín, 2016). Therefore, the increased amount of degradation products was consistent with the decrease in xylan recovery efficiency as shown in Table 2. Since 97% of xylan was degraded by pretreatment with a severity of 11.6, further increases in severity could not result in a considerable increase in the amount of degradation products from xylan. On the contrary, higher temperatures may favor further degradation of by-products into gaseous compounds, resulting in decreased amounts of degradation products in the liquor (Figure 1).

LHW pretreatment (170 °C for 5-15 min) with low severity (7.3-7.8) obtained cellulose, xylan and lignin contents of 44-49%, 19-20%, and 17%, respectively, which were similar to those of untreated giant reed (Table 2). LHW pretreatment (190 °C for 5-15 min) with higher severity (9.2-9.7) led to significantly higher cellulose contents (58-59%), lower xylan contents (10-16%), and comparable lignin contents (16-18%) to those of untreated giant reed (Table 2). LHW pretreatment (210 °C for 5-15 min) with further increased severity (11.1-11.6) achieved dramatically higher cellulose contents (65-70%) and lower xylan contents (2-3%), with a slight decrease in lignin contents (15-16%) (Table 2). When severity of LHW pretreatment was increased to 13.0-13.5 (230 °C for 5-15 min), cellulose contents decreased to 55-67%, lignin contents maintained at 14-18%, while the xylan content became zero, indicating complete xylan removal (Table 2). Alkaline pretreatment with different NaOH loadings (1-20 g/L) generally resulted in limited changes in cellulose and xylan contents of giant reed, but caused considerable decreases in lignin content (Table 2). The giant reed pretreated with 20 g/L NaOH for 24 h showed a lower lignin content (11%), and comparable cellulose (47%) and xylan (18%) contents to those pretreated with NaOH at lower loadings (Table 2).

3.2. Effect of pretreatment on glucose yield from enzymatic hydrolysis of giant reed

As shown in Figure 2, both LHW pretreatment and alkaline pretreatment improved the glucose yield from giant reed. For LHW pretreatment of giant reed, glucose yield generally increased with severity at low temperatures (170-190 °C), while it decreased with severity at high temperatures (190-230 °C) (Figure 2a). The highest glucose yield obtained by LHW pretreatment (190 °C, 15 min) was 47%, which was 2.4-fold as high as that of untreated giant reed biomass (Figure 2a). Pretreatment time (5-15 min) had no significant ($p > 0.05$) effect on glucose yield (Figure 2a). For alkaline pretreatment of giant reed, glucose yield increased with NaOH concentration, and pretreatment with 12 and 20 g/L of NaOH obtained the highest glucose yields (about 58%) (Figure 2b). The glucose yield from giant reed that was pretreated with 20 g/L NaOH was 25% higher than the highest level of LHW pretreated giant reed (Figure 2).

Xylan removal is believed to be the major mechanism for improvement of enzymatic digestibility of lignocellulosic biomass by LHW pretreatment. In previous studies on LHW pretreatment of soybean straw, corn stover, and wheat straw, enzymatic digestibility was found to be correlated to xylan removal (Kabel et al., 2007; Wan et al., 2011; Yang and Wyman, 2004). In this study, enzymatic digestibility increased with xylan removal for LHW pretreatment at 170-190 °C, then decreased with xylan removal at higher temperatures of 210-230 °C (Figure 3a). In contrast, alkaline pretreatment depends on lignin removal which makes the cellulose more accessible to hydrolytic enzymes (Wan et al., 2011). As shown in Figure 3b, the enzymatic digestibility of alkaline pretreated giant reed was correlated with lignin removal. This result was consistent with other studies on alkaline pretreatment of corn stover, in which higher enzymatic digestibility was generally correlated with higher lignin removal (Cui et al., 2012).

3.3. Effect of pretreatment on methane production from AD of giant reed

As shown in Figure 4a, daily methane yields of giant reed pretreated at 170 °C were similar to that of untreated giant reed during the 30 days of L-AD. Giant reed pretreated at 190 °C obtained a higher maximum daily methane yield (31 L/kg VS/d) than those of untreated (18 L/kg VS/d) or pretreated at 170 °C (21 L/kg VS/d) (Figure 4a). Giant reed pretreated at higher temperatures (210 and 230 °C) showed a maximum daily methane yield of 26 L/kg VS/d on the 12th day, which was 6 days later than untreated and treated (170 and 190 °C) giant reed (Figure 4a). Delayed methane production from giant reed pretreated at 210 and 230 °C was also observed based on methane content during AD (Figure 4c). Methane contents of biogas from untreated giant reed and those pretreated at 170 and 190 °C increased to 50% in 6 days during AD, while those pretreated at 210 and 230 °C reached 50% in 12 days (Figure 4c). Since the pretreated giant reed was washed prior to AD, it is unlikely that the delayed methane production was caused by inhibitors generated during LHW pretreatment at high temperatures. One possible reason is that readily degradable compounds, such as hemicellulose (or xylan), in the giant reed biomass had been mostly or completely removed after LHW pretreatment at 210 or 230 °C (Table 2), and the pretreated biomass showed decreased digestibility.

During L-AD of giant reed pretreated with different concentrations of NaOH, daily methane yield peaked in 6-9 days, and maximum daily methane yield generally increased with NaOH concentration, except that 1g/L of NaOH did not improve the methane production (Figure 4b). Methane contents of biogas from alkaline pretreated giant reed increased to 50% in 6-9 days (Figure d). Giant reed pretreated with 20 g/L NaOH achieved the highest daily methane yield of 35 L/kg VS/d, which was nearly two times that of untreated giant reed (Figure 4b). These results

are also consistent with the increase of glucose yield with NaOH concentration as shown in Figure 2b.

Figure 5 shows the effect of pretreatment on cumulative methane yield from giant reed. The untreated giant reed had a cumulative methane yield of 217 L/kg VS, which is comparable to that reported by Di et al. (2014). LHW pretreatment at different temperatures (170-230 °C) for 10 min obtained a 7-31% increase in cumulative methane yield. Similar results were also reported in a previous study, in which methane yield from giant reed was increased by 23% after LHW pretreatment at 180 °C for 10 min (Di Girolamo et al., 2013). Alkaline pretreatment with different NaOH concentrations (1-20 g/L) for 24 h also improved cumulative methane yield from AD of giant reed biomass (Figure 3). However, among different pretreatment conditions evaluated in this study, only alkaline pretreatment with 20 g/L NaOH resulted in a significant ($p < 0.05$) increase (63%) in cumulative methane yield. In a previous study in which giant reed was pretreated with 2, 4, and 6 g/L of NaOH, cumulative methane yields of 216, 229, and 246 L/kg VS, respectively, were obtained (Di Girolamo et al., 2014). In this study, higher NaOH concentrations (12 and 20 g/L) achieved higher cumulative methane yields of up to 355 L/kg VS. This is the second highest reported methane yield from untreated or pretreated giant reed biomass, following that (392 L/kg VS) reported by Ragaglini et al. (2014) and Ge et al. (2016).

It should be noted that methane yield during the AD process depends on characteristics of inoculum. The low C/N ratio (10.2) of inoculum is crucial for AD of giant reed, since giant reed had a C/N ratio (71.3) that was much higher than the commonly recommended C/N ratio of 20-30 for AD (Table 1) (Xu et al., 2013). Besides, the inoculum also provided buffer capacity to maintain a proper pH during the AD process. In addition, the inoculum can also affect the AD process due to microbial characteristics. It has been reported that dairy waste effluent had higher

numbers of cellulolytic and xylanolytic bacteria, and achieved a higher methane yield than municipal sewage sludge effluent at a relatively low F/I ratio (Xu et al., 2013). Therefore, the CH₄ yield from giant reed may possibly be further improved by using a different effluent, such as dairy waste effluent, as an inoculum.

3.4. Net energy production and cost-efficiency analysis

Untreated giant reed can generate 3,859 kJ/kg initial TS of electrical energy via AD with CHP. If the cost of giant reed biomass production is \$44/tonne TS, the net benefit of giant reed-based electricity via AD with CHP will be \$68/tonne initial TS. It was expected that pretreatment can improve the net benefit by increasing the methane yield. However, pretreatment can cause dry matter loss and additional energy and chemical inputs, which can offset the improvement in methane yield. As shown in Table 3, the energy outputs of LHW pretreated giant reed (2,361-3,698 kJ/kg initial TS) are lower than that of untreated giant reed (3,859 kJ/kg initial TS) due to the dry matter loss during LHW pretreatment (Table 2). Moreover, LHW pretreatment requires 6,090-8,610 kJ/kg initial TS for heating up the feedstock, resulting in negative net biogas energy and electrical generation benefits (Table 3). Therefore, LHW pretreatment under the studied conditions is not feasible for electricity generation from giant reed biomass via AD with CHP.

Giant reed pretreated by 1 g/L of NaOH had a potential energy output of 3,800 kJ/kg initial TS, which is still lower than that of untreated giant reed (3,859 kJ/kg initial TS) (Table 3). Higher energy outputs (4,210-4,918 kJ/kg initial TS) can be obtained by alkaline pretreatment with 5-20 g/L NaOH. The net electricity production and benefit of electricity generation are 3,800-4,918 kJ/kg initial TS, and \$110-142/kg initial TS, respectively (Table 3). Alkaline pretreatment with 5-20 g/L NaOH can achieve a 9-27% increase in net electricity production and

benefit of electricity generation from giant reed biomass via AD with CHP (Table 3). Therefore, alkaline pretreatment appeared to be more suitable than LHW pretreatment in terms of net electrical energy production.

The net benefit of electricity generation from giant reed biomass with alkaline pretreatment was \$8-61/tonne initial TS, which is lower than that of non-pretreatment due to the additional cost for NaOH input (Table 3). As a result, reuse of NaOH for the next batch of alkaline pretreatment is crucial in order to reduce the cost for chemical input and improve the net benefit compared to non-pretreatment. However, further studies are still needed to evaluate the effect of alkaline liquor reuse on performance of alkaline pretreatment.

4. Conclusion

Using the same giant reed biomass, alkaline pretreatment recovered more dry matter and achieved a 25% higher glucose yield than LHW pretreatment under optimal conditions for each method. Alkaline pretreated giant reed obtained higher methane yields (up to 355 L/kg VS) than those from giant reed untreated or pretreated with LHW. Electricity generation from giant reed biomass via AD with CHP can obtain positive net biogas energy production and net electricity generation benefit. Alkaline pretreatment is more suitable than LHW pretreatment for improving net electrical energy production, while alkaline liquor reuse is necessary in order to improve the net benefit.

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 476

Table 1 Characteristics of untreated giant reed and inoculum for anaerobic digestion (AD)

Parameters	Giant reed	Inoculum for AD
DM, %	91.0 ± 0.4	6.94 ± 0.01
ODM, %	89.7 ± 2.1	56.8 ± 0.1
TN, %DM	0.64 ± 0.01	3.83 ± 0.07
TC, %DM	45.5 ± 0.47	39.1 ± 0.5
C/N	71.3 ± 0.29	10.2 ± 0.1
pH	5.57 ± 0.03	8.40 ± 0.07
Extractives, %DM	11.8 ± 0.6	14.1 ± 0.9
Cellulose, %DM	41.5 ± 2.6	1.11 ± 0.05
Xylan, %DM	20.5 ± 0.65	ND
Lignin, %DM	18.4 ± 1.4	NA
Crude protein, %DM	4.06 ± 0.66	ND
NH ₃ -N, %DM	0.06 ± 0.02	5.25 ± 0.52

DM: dry matter; ODM: organic dry matter; ND: not detectable; NA: not applicable

Table 2 Comparison of liquid hot water and alkaline pretreatment of giant reed on composition of pretreated biomass and mass recovery after pretreatment process

Pretreatment conditions	Severity	Dry matter recovery (%)			Cellulose recovery (%)	Xylan recovery (%)	Lignin removal (%)	Composition of pretreated solids (%)		
		Solid	Liquor	Total				Cellulose	Xylan	Lignin
Liquid hot water pretreatment										
170 °C, 5 min	7.3	84.5	13.9	98.4	88.4	83.7	21.9	43.5	20.3	17.0
170 °C, 10 min	7.6	80.0	15.3	95.4	85.0	73.5	24.8	43.8	18.8	17.3
170 °C, 15 min	7.8	79.7	15.4	95.1	94.1	76.4	24.8	48.8	19.7	17.4
190 °C, 5 min	9.2	73.7	19.8	93.5	93.3	63.8	35.9	57.8	16.2	16.0
190 °C, 10 min	9.5	68.8	21.6	90.4	89.1	47.9	32.9	58.7	13.2	17.9
190 °C, 15 min	9.7	63.6	25.3	88.9	91.5	28.7	42.9	59.1	10.4	18.0
210 °C, 5 min	11.1	58.2	22.4	80.5	95.9	8.2	54.0	69.8	2.9	14.5
210 °C, 10 min	11.4	58.0	21.8	79.4	95.7	6.7	49.7	69.9	2.4	15.9
210 °C, 15 min	11.6	58.0	19.1	77.0	85.7	3.5	49.1	64.9	1.6	16.1
230 °C, 5 min	13.0	56.1	19.7	75.8	84.6	0.0	57.5	62.6	0.0	14.0
230 °C, 10 min	13.3	55.7	18.4	74.2	86.4	0.0	48.7	66.9	0.0	16.9
230 °C, 15 min	13.5	55.2	18.4	73.5	72.2	0.0	46.1	54.9	0.0	18.0
Alkaline pretreatment										
1 g/L NaOH, 24 h	-	86.6	-	-	86.1	81.3	31.5	41.2	19.2	14.5
3 g/L NaOH, 24 h	-	85.9	-	-	88.9	86.5	32.0	42.9	20.6	14.6
5 g/L NaOH, 24 h	-	82.5	-	-	81.0	77.2	29.1	40.7	19.2	15.8
7 g/L NaOH, 24 h	-	82.4	-	-	94.4	85.8	36.8	47.8	21.3	14.1
12 g/L NaOH, 24 h	-	76.7	-	-	90.6	83.4	46.2	49.0	22.3	12.9
20 g/L NaOH, 24 h	-	74.9	-	-	84.5	66.9	54.7	46.8	18.3	11.1

Table 3 Effect of liquid hot water pretreatment and alkaline pretreatment on net benefit of electricity generation using biogas from AD of giant reed

Pretreatment conditions	Total biomass energy production (kJ/kg TS)	Energy input for pretreatment (kJ/kg TS)	Net biogas energy production (kJ/kg TS)	Benefit of electricity generation (\$/tonne TS)	Cost of biomass production (\$/tonne TS)	Cost for Chemical input (\$/tonne TS)	Net benefit (\$/tonne TS)
Liquid hot water pretreatment							
170°C, 10min	3,698	6,090	Negative	Negative	44	0	Negative
190°C, 10min	3,593	6,930	Negative	Negative	44	0	Negative
210°C, 10min	2,940	7,770	Negative	Negative	44	0	Negative
230°C, 10min	2,361	8,610	Negative	Negative	44	0	Negative
Alkaline pretreatment							
1g/L NaOH, 24h	3,800	0	3,800	110	44	5	61
5g/L NaOH, 24h	4,210	0	4,210	122	44	23	55
12g/L NaOH, 24h	4,485	0	4,485	130	44	54	32
20g/L NaOH, 24h	4,918	0	4,918	142	44	90	8
Non-pretreatment							
-	3,859	0	3,859	112	44	0	68

Note: All values are based on initial TS of giant reed biomass.

Figure 1. Degradation products formed during LHW pretreatment of giant reed

Figure 2. Effect of (a) LHW pretreatment and (b) alkaline pretreatment on glucose yield from giant reed

Figure 3. Correlation between (a) xylan removal and enzymatic digestibility of giant reed pretreated by LHW, and (b) lignin removal and enzymatic digestibility of giant reed pretreated by alkaline

Figure 4. Daily methane yield from L-AD of (a) LHW pretreated and (b) alkaline pretreated giant reed biomass, and effect of (c) LHW pretreatment and (d) alkaline pretreatment on methane content during the L-AD process

Figure 5. Effect of LHW and alkaline pretreatment on cumulative CH₄ yield by 30-day L-AD of giant reed. Means that do not share a letter (i.e., a or b only) are significantly different at $p < 0.05$.