Assessments of *Erianthus arundinaceus* as a Potential Energy Crop for Bioethanol and Biomethane Production

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Six varieties of *Erianthus arundinaceus* were assessed as potential energy crops and compared with switchgrass. Bioethanol and biomethane were produced as well. The chemical composition, elemental composition, and heating values were close to those of switchgrass except for a higher cellulose content. All varieties scored higher than 110, suggesting excellent potential as an energy crop. Pretreatment resulted in almost complete hydrolysis and achieved a higher glucose yield. In particular, E33 and E19 yielded 337.4 g and 332.4 g glucose, respectively, from 1.0 kg of feedstock compared with 312.1g/kg for switchgrass. E33, E49, and E19 exhibited a higher ethanol yield of 144.2 g/kg, 146.9 g/kg, and 149.6 g/kg feedstock, respectively, at a solids loading of 15%. No obvious influences could be observed on anaerobic digestion performances. A 16.3%, 14.6%, 14.0%, and 13.1% higher yield on cumulative methane could be obtained from E17, E23, E33, and E6, respectively, compared to switchgrass. Bioethanol and biomethane could be maximally obtained from E19 (6820 kg/ha) and E17 (3916 m^3/ha), respectively. Thus, they are specially recommended for bioethanol and biomethane production, respectively. E33 can be suggested as a flexible variety for yielding relatively high bioethanol (6008 kg/ha) and biomethane (3409 m^3/ha).

Keywords: Erianthus arundinaceus; Energy crop; Bioethanol; Anaerobic digestion

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INTRODUCTION

Many sources of motivation, such as reduction of greenhouse gas emissions, rising energy demands, and the desired independency from oil imports, are promoting renewable energy production (Scarlat *et al*. 2015; Rahman *et al*. 2017). Energy security and declining fossil fuel resources have especially stimulated the advancement of sustainable and renewable technologies that utilize cheap biomass. Moreover, biomass is available abundantly on earth and is the most common component to provide renewable energy without dramatic changes in infrastructure and consumption technologies (Leitner and Lindorfer 2016; Al-Hamamre *et al*. 2017).

Biomass can be converted into various types of biofuels or energy *via* thermal, physical, and biological processes (Gonzalez-Salazar *et al*. 2016). Bioethanol and biomethane produced from biomass are two popular conversion processes, due to their sustainable alternatives to gasoline and natural gas, respectively, and they are widely accepted in many countries. Traditionally, bioethanol is produced from sugary or starchy

feedstocks, such as sugar cane, maize, and other grains, but these feedstocks cannot meet the global demand of bioethanol production due to their primary value as food and feed (Gupta and Verma 2015). Biomethane can be produced from animal manure, activated sludge, or any type of organic waste and by-product (Weiland 2010). Producing bioethanol and biomethane from the lignocellulosic parts of energy crops solves the problem of competition for bioethanol production and food demands and also offers additional options for feedstocks to produce biomethane.

Lignocellulosic energy crops with high biomass yield, strong tolerance to abiotic stresses, and disease resistance can be produced on marginal land, which makes energy crops attractive compared with traditional waste biomass (Khanna *et al*. 2017). Moreover, the high benefits have prompted agricultural producers to use energy crops to produce biogas, as they are more productive in terms of methane produced per kg of biomass, allowing medium-size biogas plants to be established (Corno *et al*. 2015). However, excessive unitary cropping patterns may cause serious issues on biodiversity conservation once energy crops are employed long-term as the biomass sources for energy refinery. Thus, seeking potential plant varieties to diversify energy crops is a possible solution to biodiversity conservation (Wang *et al*. 2015).

Erianthus arundinaceus is a warm-season, perennial species native to China and other southern and southeastern Asian nations in the tropical area. It plays an important role in breeding high-yield and high-sugar content sugarcane because it is closely related to *Saccharum officinarum* L. The species is related to taxa in *Miscanthus*, *Narenga*, *Saccharum,* and *Sclerostachya,* so it is considered a member of the "sugarcane complex" (Zhang *et al*. 2013). *E. arundinaceus* is characterized by its tall stalk (height range of 2 m to 4 m), dense clusters, strong rooting and tillering rate, wide adaptability, and high resistance to abiotic and biotic stresses. In addition, the high biomass yield (approximately 40 t/ha, dry weight) makes it a potential energy crop for bioenergy production (Yan *et al*. 2016). Recent investigations on *E. arundinaceus* have been mainly focused on variety screening and cultivation and on clarifying the key genetic characteristics (Yamamura *et al*. 2013; Augustine *et al*. 2015; Tsuruta *et al*. 2017). However, the actual performances for converting *E. arundinaceus* to typical energy products lacks some necessary assessments and investigation.

In this study, approximately 200 wild varieties from different areas of China were collected. Six varieties of *E. arundinaceus* were selected according to their biomass yield and cultured for potential energy crops. Two typical bioenergy products, bioethanol and biomethane, were investigated to assess the potential energy conversion efficiency. A widely-accepted energy crop of switchgrass was employed as a control for comparison.

EXPERIMENTAL

Erianthus arundinaceus, Inoculum, Enzyme, and Yeast

Six varieties of *Erianthus arundinaceus* were collected by the Sichuan Academy of Grassland Science, China, and they were named E17, E33, E49, E19, E23, and E6. The harvested biomass was air-dried and ground to less than 2 mm. The prepared biomass was stored at 4 °C before composition determination, anaerobic digestion, pretreatment for enzymatic hydrolysis, and subsequent ethanol fermentation. A recognized energy crop—the Alamo variety of switchgrass (*Panicum virgatum* L.)—was cultured for a comparison.

The inoculum for batch anaerobic digestion was obtained from the Biogas Institute

of Ministry of Agriculture, Chengdu, China. The inoculum was pre-incubated for 15 days at a mesophilic temperature to deplete the residual biodegradable organic materials (degasification). The basic characteristics of inoculum were total solids (TS) of 3.7%, volatile solids (VS) of 52.2% (dry basis), ash content of 47.8% (dry basis), cellulose of 9.3% (dry basis), hemicellulose of 4.2% (dry basis), lignin of 19.4% (dry basis), C/N of 6.6 , and pH 7.42.

Enzymatic hydrolysis and simultaneous saccharification and fermentation (SSF) were employed to estimate the glucose yield and ethanol yield, respectively. The enzyme for hydrolysis and SSF, Cellic CTec2, was obtained from Novozymes (Beijing, China). The active dry yeast, *Saccharomyces cerevisiae* (purchased from Angel Yeast Co., Ltd., Yichang City, China), was employed for SSF; this yeast is characterized by high-speed fermentation and excellent resistance to ethanol and temperature. Prior to fermentation, dry yeast was activated in YPG liquid medium (10 g/L yeast extract + 20 g/ L peptone + 20 g/L glucose) at 37 °C for 2.0 h at 150 rpm. After activation, yeast cells were centrifuged at 5500 rpm and 4 °C for 5 min. The cell slurry was washed with sterile distilled water and centrifuged under the same conditions. The washing process was repeated 3 to 4 times until the supernatant was transparent and colorless. Yeast concentration was determined by a spectrophotometer at 600 nm. The calibration curve was constructed with a standard solution of dry yeast (10 g/L). The amount of yeast required for inoculation during fermentation was calculated according to the calibration curve.

Batch Anaerobic Digestion

One-liter glass bottles were used as anaerobic digestion reactors, and the total working weight was 800 g for each batch. The substrate loading for all feedstocks was controlled at 30 g TS/kg, and the seeding sludge inoculation was 15 g TS/kg. All digestion runs were investigated in triplicate. Inoculum alone was also digested as a control. After the substrates and inoculum were loaded, each bottle was sealed with a rubber stopper, and the headspace was flushed with pure N_2 for 2.0 min. The bottles were incubated at a mesophilic temperature (35.0 ± 1.0 °C) in a water bath. Anaerobic digestion continued for 50 days. The biogas production was determined daily *via* the water displacement method, and to determine the composition, 1.0 mL of biogas was sampled using a syringe.

Pretreatment

To assess the bioethanol production for the lignocellulosic biomass, pretreatment is a key step for the subsequent SSF. A recently developed pretreatment method of concentrated phosphoric acid plus hydrogen peroxide (PHP) was selected due to the efficiency of hemicellulose and lignin removal, high cellulose recovery, and adaptability to various typical lignocelluloses (Wang *et al*. 2014). The pretreatment conditions were 40 °C, 2.0 h, and H_3PO_4 proportion of 70.2% (H_2O_2 proportion of 5.2%), which was optimized based on the grassy biomass of the wheat straw, as described previously (Qiu *et al*. 2017). The pretreatment was stopped by a rapid dilution with ethanol (220 mL, 95% v/v). The precipitated solid was filtered and washed with ethanol 3 to 5 times. The recovered liquor containing a mixture of the ethanol and H_3PO_4 was separated by distillation. The extracted ethanol was recycled to stop the pretreatment and precipitate the pretreated substrates, whereas the separated H_3PO_4 was used to recover lignin that was dissolved or degraded during the pretreatment. The ethanol-washed substrates were washed with distilled water 2 to 3 times and stored at -20 °C before analysis and SSF.

Enzymatic Hydrolysis and SSF

Enzymatic hydrolysis of the pretreated substrates was performed with 2% (dry basis) solids loading to evaluate the maximum glucose yield from the investigated feedstocks. The SSF with a solids loading of 2% or 15% was used to assess the potential ethanol yield or the ethanol production that appeared in practice, respectively. Enzymatic hydrolysis was performed in a 100 mL bottle with a working volume of 20 mL at 50 °C. The SSF was performed in a 10 mL vial with a working volume of 2 mL, and 18 vials were employed for each run. The enzyme and yeast loading was set at 60 mg protein/ g cellulose and 3%, respectively. The fermentation was performed at 38 °C in an incubation shaker at 170 rpm for 120 h. Three vials of each sample were withdrawn at 12 h, 24 h, 48 h, 72 h, 96 h, and 120 h after fermentation and heated at 100 °C for 5 min to inactivate the enzyme and yeast. The samples were centrifuged at 1.3×10^5 rpm and 4 °C for 10 min. The supernatant was stored in -20 °C before determining the sugar and ethanol concentration.

Analysis

Chemical composition

The original feedstocks and pretreated substrates were analyzed for Klason insoluble lignin and carbohydrates using the TAPPI T222 om-11 (2011). The hydrolysate from the Klason analysis was retained and analyzed for soluble lignin using a UV spectrophotometer at 205 nm. The glucose and xylose in the hydrolysate were measured using a high-performance liquid chromatography (HPLC) (Flexar, PerkinElmer, Waltham, MA, USA) with a refractive index detector (50 °C). The sugars were separated by a sugar column (SH1011, Shodex, Showa Denko America, Inc., New York, USA) at 60 °C using 0.05 $mol \cdot L^{-1}$ H_2SO_4 as mobile phase with a flow rate of 0.8 $mL \cdot min^{-1}$. The injection volume for the HPLC analysis was 100 μL. Lactose (~0.5 $g \cdot L^{-1}$, Sigma-Aldrich, Shanghai, China) was used as an internal standard. The obtained glucose and xylose content were used for calculating the glucan and xylan content in the substrates, which were employed to represent the cellulose and hemicellulose content in the substrates. Based on the mass of the recovered solid from pretreatments, and the determined lignin, xylan, and glucan content in the biomass, the lignin removal, hemicellulose recovery, and glucan recovery were calculated as previously described (Li *et al*. 2014).

Biogas composition

Biogas composition, including CH_4, CO_2, N_2, and H_2, was measured using gas chromatography (GC) (SP-2100A, Beifen-Ruili Instrumental Analysis Co., Ltd, Beijing, China) equipped with a molecular sieve packed stainless-steel column (TDX-01, 2.0 m in length, 3.0 mm in diameter) and a thermal conductivity detector (TCD). The temperature of the injector, oven, and detector were 50 °C, 50 °C, and 100 °C, respectively. The injection volume of the biogas sample was 1.0 mL. A standard gas consisting of 52.81% (v/v) CH_4, 32.27% (v/v) CO_2, 4.93% (v/v) N_2, and 9.99% (v/v) H_2 was used for calibrating reads from the gas chromatography. The detailed protocol for determining biogas composition was described by De La Rubia *et al*. (2009).

Ethanol concentration

Ethanol was analyzed using a GC (SP-2100A, Beifen-Ruili Instrumental Analysis Co., Ltd, Beijing, China) equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm × 0.25 μm column (Agilent, DB-WAX). Helium was employed as the carrier gas with flow rate of 20 mL/min. The temperatures of the injection unit and FID detector were

175 °C and 250 °C, respectively. The oven was heated to 45 °C for 2.5 min, and the temperature was raised to 110 °C at a rate of 20 °C/min and later held at 110 °C for 2 min. The internal standard method was applied, in which the ethanol (chromatographic grade) and butanol (0.5 g/L) were used for the standard curve and internal standard substance separately. Samples of 0.1 mL were injected directly into the column. Measurements were taken from standard curves, and the final results were the average of two repetitions.

RESULTS AND DISCUSSION

E. arundinaceus Composition and Assessment as a Potential Energy Crop

The chemical and elemental composition of *E. arundinaceus* and switchgrass is listed in Table 1. The determined composition of switchgrass was similar to previously reported results (Capecchi *et al*. 2016). Cellulose, the most abundant biopolymer, is a high molecular weight polymer composed of beta-(1-4)-D-glucopyranose units. The cellulose content in the six varieties of *E. arundinaceus* ranged from 32.3% to 35.5%, which was higher than that of the typical energy crop, switchgrass (Alamo). This result indicated a higher potential for glucose release in energy conversion. Hemicellulose is the second major carbohydrate component in cell walls, and it is a combination of different monosaccharides including glucose, mannose, galactose, xylose, arabinose, and uronic acids. Xylan was the dominant fraction in the hemicellulose of *E. arundinaceus*, and its content ranged between 19.3% and 22.5%, which was similar to switchgrass (19.8%).

Lignin is the third main component in the cell walls of lignocellulose biomass. It is an amorphous polyphenolic cross-linked biopolymer that is generally associated with cellulose and hemicellulose. It acts as a primary binder for cellulosic fibers and also provides a defense against microbial and fungal attack of the cellulose fibers. The lignin content in the six varieties was higher than that of switchgrass, which may potentially increase the difficulty of bioenergy conversion in a biological route due to the hindrance of the existing lignin (Murciano Martínez *et al*. 2015). As a non-utilizable fraction for bioenergy conversion, ash plays a negative role in the thermo-chemical and biological conversion processes, and will need to be properly removed (David and Ragauskas 2010). The ash content in *Erianthus arundinaceus* was very similar to that of switchgrass and lower than the traditional annual grassy-biomass, such as agricultural straw (Chandra *et al*. 2015). The relatively low ash may be beneficial to biological conversion or to direct combustion for heating (Sheng and Azevedo 2005; Kim and Day 2011). However, these values are higher than the ash content of other woody energy crops, such as hybrid poplar (Dou *et al*. 2016). In addition, the extractives in *E. arundinaceus* were lower than those of switchgrass, which may potentially affect anaerobic digestion due to the easily degradable fraction in the extractives (Yan *et al*. 2010).

Knowing a material's elemental composition is useful in determining the potential of a given bioresource for biofuels and biopower applications (David and Ragauskas 2010). The elemental analysis for switchgrass was similar to previous reports (Lemus *et al*. 2002; David and Ragauskas 2010), and the content in different varieties of *E. arundinaceu*s was close to that of switchgrass and hybrid poplar, another potential biofuel feedstock (Bridgeman *et al*. 2007; David and Ragauskas 2010). The heating value of a feedstock is also important in bioenergy conversion by thermo-chemical pathways. The values of the *E. arundinaceu*s cultivars are presented in Table 1.

Table 1. Basic Composition and Heating Values of *E. arundinaceus* and *Panicum virgatum*

Variety	Chemical Composition (%, wt/wt)					Elemental Composition (%)				High Heating Value ($MJ \cdot kg^{-1}$)
	Cellulose	Hemi-cellulose	Lignin	Ash	Extrac-tives	C	H	N	O	
Alamo	30.3 ± 0.6	19.8 ± 0.5	19.6 ± 0.1	4.0 ± 0.1	16.3 ± 1.1	44.0	6.01	0.67	45.3	17.7 ± 0.01
E17	35.5 ± 2.5	22.5 ± 1.4	20.1 ± 0.1	4.8 ± 0.1	12.4 ± 0.6	45.1	5.78	0.72	43.5	18.0 ± 0.01
E33	32.3 ± 0.3	19.5 ± 0.2	21.1 ± 0.2	3.7 ± 0.1	11.0 ± 1.0	44.7	5.78	0.83	45.0	17.7 ± 0.01
E49	34.3 ± 0.3	20.6 ± 0.2	23.0 ± 0.9	3.7 ± 0.1	9.1 ± 0.4	45.0	5.75	0.37	45.4	17.7 ± 0.00
E19	33.3 ± 1.1	19.3 ± 0.4	21.8 ± 0.3	3.8 ± 0.0	7.8 ± 0.5	45.8	6.27	0.52	43.6	18.8 ± 0.00
E23	32.6 ± 2.2	19.7 ± 1.1	22.6 ± 0.0	4.1 ± 0.1	7.8 ± 0.9	44.8	5.76	0.80	44.5	17.7 ± 0.00
E6	35.0 ± 0.4	20.5 ± 0.1	20.5 ± 0.1	4.5 ± 0.1	9.3 ± 1.2	44.7	5.57	0.37	45.2	17.4 ± 0.01

The high heating value varied from 17.4 $MJ \cdot kg^{-1}$ to 18.8 $MJ \cdot kg^{-1}$, which was almost equal to that of the switchgrass and other grassy feedstocks, such as reed canary grass, with a value of ~18 $MJ \cdot kg^{-1}$, and lower than that of hybrid poplar (approximately 19 $MJ \cdot kg^{-1}$) (Lemus *et al*. 2002; Dien *et al*. 2006).

*E. arundinaceu*s was evaluated as a potential energy crop by considering chemical composition, biomass, ecological adaptability, and heating value (McKendry 2002). The detailed evaluation process is described in the Supplementary Information. Based on the criteria for evaluating the energy crop, a score higher than 75 is regarded as an excellent crop that suits bioenergy production; as a typical energy crop, switchgrass had a score of 83.8, as presented in Fig. 1. The scores of the six varieties *of E. arundinaceus* were all higher than 110, which was attributed to their extremely high biomass yield (Table 4). This result indicated that *E. arundinaceus* is a potential energy crop. The scores of the six varieties of *E. arundinaceus* were also higher than those of other typical energy crops, such as hybrid aspen (81.8), elephant grass (76.6), *Miscanthus* (76.3), and short-rotation-coppice willow (75.2) (Wang *et al*. 2015). However, the actual performance for converting *E. arundinaceus* to typical bioenergy has not been investigated.

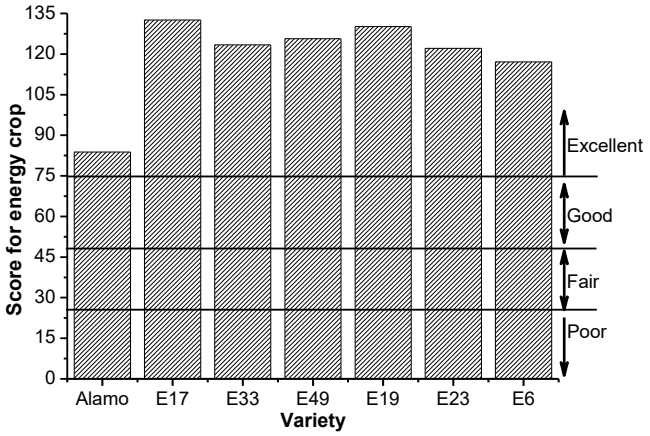

Fig. 1. Criteria for evaluating *Erianthus arundinaceus* as a potential energy crop. The score of ≥ 75 suggests that it is an excellent energy crop; the scores of 50 to 75 (including 50), 25 to 50 (including 25), and < 25 indicates good, fair, and poor or not applicable, respectively.

Enzymatic Saccharification of *E. arundinaceus* after PHP Pretreatment

Converting lignocellulosic biomass to bioethanol *via* the biological route is more difficult than with starch or sugar biomass, which can be easily hydrolyzed or utilized directly. To release the fermentable sugar from lignocellulosic biomass, pretreatment is a crucial step. Although many pretreatment technologies have been developed to break the recalcitrance of biomass, fermentable carbohydrates loss is an unavoidable fact that may seriously affect bioethanol production. The PHP method has been developed to pretreat lignocellulosic biomass, resulting in more than 80% cellulose recovered from feedstocks, and the recovered solid can be effectively enzymatically hydrolyzed (with a cellulose-glucose conversion higher than 90%). Thus, PHP can be employed to pretreat *E. arundinaceus* and evaluate the glucose and ethanol production. The chemical composition of the pretreated biomass after PHP pretreatment is listed in Table 2.

Table 2. Chemical Composition after PHP Pretreatment

Variety	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Solid Recovery (%)	Cellulose Recovery (%)	Hemicellulose Removal (%)	Lignin Removal (%)
Alamo	70.5 ± 1.64	10.3 ± 1.62	10.6 ± 1.05	39.8	92.7	79.2	78.4
E17	64.9 ± 0.17	11.7 ± 0.87	11.8 ± 0.89	35.7	65.5	81.7	79.0
E33	78.2 ± 2.00	10.5 ± 2.07	9.5 ± 1.11	38.8	93.9	79.1	82.5
E49	73.2 ± 0.98	11.5 ± 1.81	9.5 ± 1.23	39.5	84.2	78.0	83.7
E19	78.2 ± 0.74	10.4 ± 0.88	8.3 ± 0.87	38.9	91.5	79.0	83.5
E23	72.3 ± 0.58	13.6 ± 1.09	10.9 ± 1.54	35.2	78.5	75.2	84.5
E6	74.0 ± 0.93	12.2 ± 2.88	10.1 ± 1.79	38.0	80.4	77.2	81.2

The hemicellulose and lignin contents decreased greatly as these feedstocks were pretreated with PHP, and the cellulose content increased from 30% to 36% to 65% to 78%. However, it was unavoidable that some of the cellulose was lost because it is very hard to make the pretreatment conditions match the biomass well due to the differences in its structure and composition. The cellulose recovery of switchgrass (Alamo) was 92.7%, which was close to the results of PHP pretreatment on most types of lignocellulosic feedstocks. The cellulose recovery from E33 and E19 was 93.9% and 91.5%, respectively, which was comparable to that of Alamo, suggesting that these two varieties respond to PHP pretreatment in a similar way to switchgrass due to similar micro-structures. Less than 20% cellulose loss appeared in the varieties of E49 and E6, which is acceptable in practice for ethanol production from lignocellulosic biomass. Relatively serious cellulose loss occurred in the varieties of E17 and E23, further indicating that the structure of these two varieties may be less recalcitrant than the other varieties. As two important recalcitrant fractions, hemicellulose and lignin in switchgrass were removed at levels of 79.2% and 78.4%. The lignin removal was close to the reported results on various types feedstocks, and the hemicellulose removal was lower than the reported results (Wang *et al*. 2014). Overall, the hemicellulose removal of *E. arundinaceus* was in the range of 75.2% to 81.7%, which was very close to that of switchgrass, and the lignin removal was higher than that of switchgrass. Based on these results, the investigated varieties of *E. arundinaceus* were structurally less recalcitrant in response to pretreatment. Thus, it is predicted that they would display excellent efficiency in glucose release by enzymatic hydrolysis.

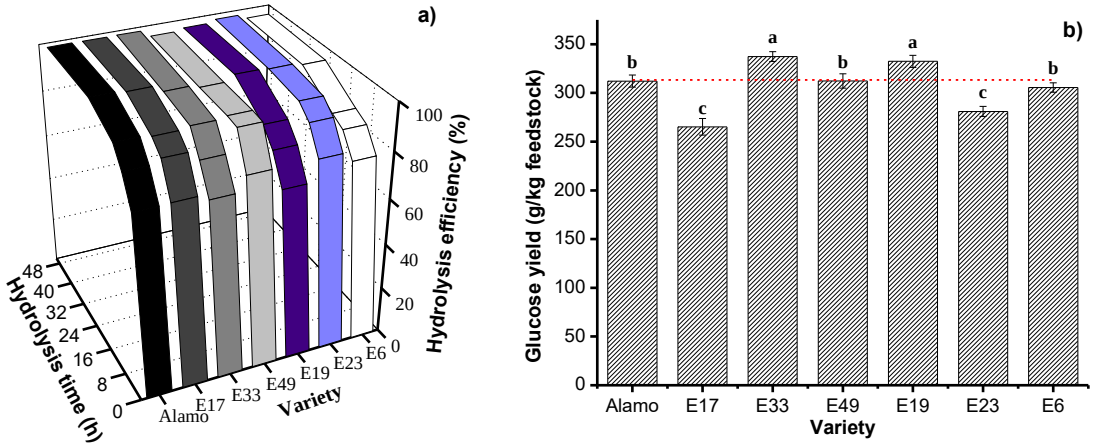

Fig. 2. Enzymatic hydrolysis and glucose yield of *E. arundinaceus* after PHP pretreatment

To assess glucose release from pretreated *E. arundinaceus*, enzymatic hydrolysis was performed with excess enzyme loading (60 mg protein/g cellulose) and with 2% solid loading. The hydrolysis behaviors are plotted in Fig. 2a, and the glucose yield from the pretreated feedstocks in these conditions was regarded as the maximum potential glucose yield. The pretreated switchgrass (Alamo) was completely hydrolyzed within 24 h. The results for the *E. arundinaceus* variety E17 were similar to switchgrass and rapidly achieved 100% hydrolysis in 24 h, which may be related to its easy responses to PHP pretreatment (relatively low solid recovery and cellulose recovery, see Table 2). The hydrolysis of pretreated E33 also reached 100% in 48 h. For the remaining varieties, enzymatic hydrolysis of 97% to 99% was achieved in 48 h, which indicates highly efficient glucose production. These results indicated that the selected varieties of *E. arundinaceus* corresponded well to the pretreatment of PHP, and the enzymatic hydrolysis behaviors did not exhibit a significant difference ($p > 0.05$) compared with the switchgrass. As the glucose yield was calculated based on 1 kg feedstock (dry basis) (see Fig. 2b), switchgrass can yield 312.1 g/kg feedstock. In contrast, the of E33, E49, and E19 varieties yielded 337.4 g, 312.3 g, and 332.4 g glucose, respectively, from 1 kg feedstock. For variety E6, its glucose yield was 2.1% lower than that of switchgrass without significant difference ($p > 0.05$). However, a significant decrease of approximately 10% and 15% on the final glucose yield was observed in E23 and E17 compared with the switchgrass. According to the efficient hydrolysis rate of E23 and E17, the higher cellulose loss may be responsible for the low glucose yield. In sum, the investigated varieties of *E. arundinaceus* were suitable for fermentable sugar (glucose) production, especially the E33, E19, and E49 varieties.

Ethanol Conversion from *E. arundinaceus* by SSF

Ethanol production by yeast fermentation depends on the performance of fermentable sugar release, the type of feedstock, and the by-products from pretreatment, for example, the nutrients in feedstock and the inhibitors derived from pretreatment. Thus, it is necessary to evaluate the potential of ethanol production by actual fermentation, rather than the calculation based on sugar contents. In order to avoid the potential influence from yeast, enzyme, and substrate, a very low solid loading of 2% with a high enzyme loading (60 mg protein/g cellulose) and yeast loading of 3% were employed for the SSF, and the final ethanol yield is presented in Table 3.

Table 3. Ethanol Conversion from PHP-pretreated *E. arundinaceus* at a Solid Loading of 2%

Varieties	Ethanol Concentration (mg/mL)	Conversion of Cellulose to Ethanol* (%, based on the theoretic ratio)	Ethanol Yield[&] (g/kg feedstock, dry basis)
Alamo	8.7 ± 0.001 ab	100 ± 0.01[#] a	169.1 ± 0.02 a
E17	7.5 ± 0.306 c	100 ± 1.95 a	132.0 ± 1.04 c
E33	7.9 ± 0.505 bc	91.3 ± 5.57 b	157.4 ± 9.60 ab
E49	8.5 ± 0.263 ab	99.6 ± 3.09 a	163.7 ± 5.08 ab
E19	9.0 ± 0.063 a	98.8 ± 0.70 a	170.9 ± 1.21 a
E23	7.4 ± 0.055 c	96.8 ± 0.65 ab	127.6 ± 0.94 c
E6	9.2 ± 0.329 a	100 ± 3.84[#] a	172.4 ± 6.13 a

Note: [*] The conversion of cellulose to ethanol is calculated based on the cellulose content in the pretreated feedstock; [#] The actual calculated conversions in these two groups were 105% and 107%, respectively, which higher than 100%, and here the data were regarded as 100%; [&] The ethanol yield was calculated based on the ethanol output from 1 kg feedstock (dry basis).

After 72 h, the final ethanol concentration in the broth was 8.7 mg/mL for the switchgrass. The ethanol concentration for varieties of E19 and E6 were higher than that of switchgrass and E49 was close to that of the switchgrass without significant difference ($p > 0.05$). The amounts of ethanol produced from other varieties were in the range of 7.4 mg/mL to 7.9 mg/mL, slightly lower than the switchgrass. As for the ethanol conversion from cellulose, more than 91% conversion of cellulose to ethanol could be achieved in the pretreated *Erianthus arundinaceus*, and most varieties were very close to the switchgrass ($p > 0.05$) except for E33 ($p < 0.05$). This result indicated that PHP pretreatment did not introduce serious inhibitors to the subsequent fermentation, and the difference in the conversion of cellulose to ethanol may be attributed to the micro-structural characteristics of pretreated substrates, such as the degree of crystallinity, fiber size, and hemicellulose or lignin contents (Arantes and Saddler 2011). As the final ethanol yield from 1 kg feedstock was calculated, the yields from the varieties of E19 and E6 exhibited to be slightly higher than that of switchgrass. Although the ethanol yields from E33 and E49 were lower than that of switchgrass, the difference was not significant ($p > 0.05$). The final ethanol yields from the other two varieties of E17 and E23 were 22% and 25% lower than that of switchgrass. The E33, E49, E19, and E6 varieties exhibited excellent potential for bioethanol production.

The relatively low ethanol concentration after fermentation is another crucial issue in the higher cost of ethanol production from lignocellulosic biomass in practice. Generally, increasing the solid loading to achieve higher sugar concentration, and production of more ethanol is a widely acceptable way to solve this issue in practice. However, large amounts of substrate will definitely decrease the hydrolysis due to much lower mixing efficiency, lower contact between substrate and enzyme, nonspecific adsorption on non-cellulosic components such as lignin, and loss of the catalytic activity by shearing effects (Ramachandriya *et al*. 2013). Even though a high amount of enzyme is input for hydrolysis or fermentation at high solid loading, additional problems, such as an increased competition for the substrate sites available for hydrolysis and enzyme jamming, are involved (Várnai *et al*. 2013). Therefore, the ethanol production at lower solid loading with a large amount of enzyme and yeast can only reflect the potential of ethanol yield from biomass. However, it is prudent to check the actual productivity at a higher solid loading. Generally, an ethanol concertation higher than 40 mg/mL in the broth will curtail

the cost of ethanol distillation, and the solid loading at 15% is widely acceptable in practice (Olofsson *et al*. 2008). The SSF using the pretreated *E. arundinaceus* at 15% solid loading was performed on the varieties of E33, E49, E19, and E6, respectively, and the results are displayed in Fig. 3. The ethanol concentration in the broth can be rapidly accumulated to higher than 40 mg/mL as the SSF was performed for 24 h. The maximum ethanol concentration was achieved at 67.8 mg/mL from E19, and the other varieties were all higher than that of switchgrass (62.5 mg/mL). The difference in the ethanol yield may relate to the fiber nature after pretreatment, such as fiber length, width, and the existing pore sizes (Arantes and Saddler 2011). The fiber sizes, especially fiber length, relating to the DP (degree of polymerization), affect the viscosity, resulting in the difficulty in mixing and cellulase adsorption and desorption. The pore sizes definitely related to the accessibility of enzymes on the substrate surface (Qiu *et al*. 2017). These factors limit the glucose release, especially at higher solid loading. As for the glucose concentration during the SSF, glucose accumulation peaks could be obviously observed at the first 12 h, indicating the rapid enzymatic hydrolysis in the initial period and the glucose release was faster than that consumption of yeast. Afterwards, the glucose concentration in all groups were balanced at very low levels (< 2.0 mg/mL), suggesting the released sugar was rapidly converted by yeast. A slight increase of glucose concentration implied the yeast activity exhibited a decrease after long time work on fermentation. Overall, the residual glucose concentration after 96 h SSF in all the pretreated *E. arundinaceus* was in very low levels, even lower than that of switchgrass, potentially suggesting better conditions for yeast metabolism for ethanol release from *E. arundinaceus*. As for the ethanol conversion from cellulose in the pretreated substrates, the variety of E49 was higher than that of switchgrass, and E33, E19 and E6 were slightly lower without significant difference ($p > 0.05$). The final ethanol yield from E33, E49, and E19 was 144.2 g/kg, 146.9 g/kg, 149.6 g/kg, respectively, which were all higher than that of switchgrass (141.0 g/kg). Apparently, the ethanol yield in practice was comparable to the switchgrass. Especially the varieties of E23 and E19, even more ethanol could be potentially released once the pretreatment or the fermentation conditions were optimized further. Based on the results on the potential ethanol yield and the ethanol yield at high solid loading, the variety of E49 exhibited excellent performances on ethanol conversion efficiency and ethanol yield.

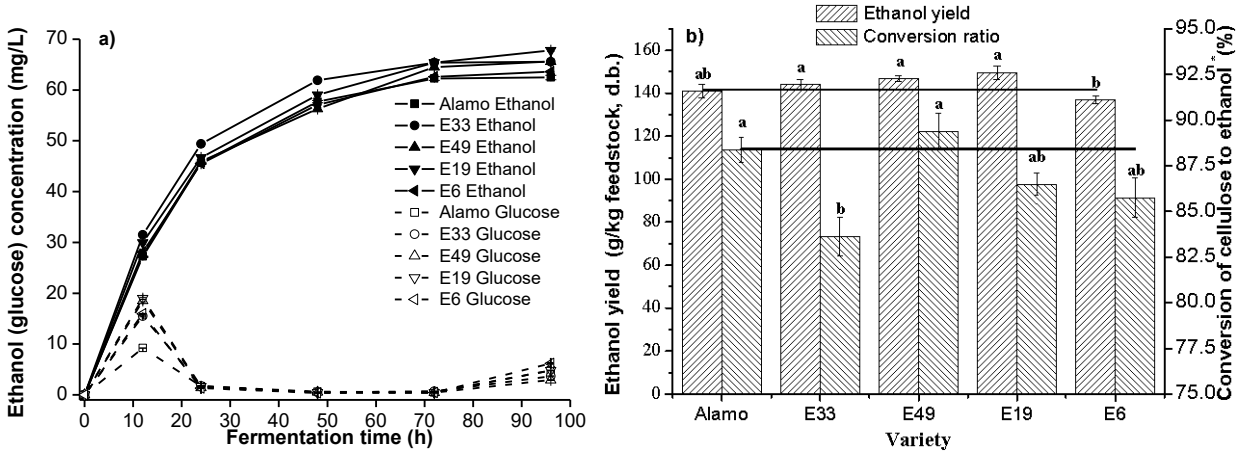

Fig. 3. Ethanol production from PHP pretreated *E. arundinaceus* at higher solid loading of 15%.
* Conversion of cellulose to ethanol is calculated based on the cellulose content in the pretreated feedstock.

Biomethane Conversion from *E. arundinaceus* by Anaerobic Digestion

As reported, 3% TS content was the critical point for anaerobic digestion of switchgrass, and a higher TS content potentially caused digestion failure (Zheng *et al*. 2015). Thus, the anaerobic digestion with 3% TS solid loading for 50 days was selected for *E. arundinaceus* in this work to avoid the potential failure during the biogas estimation, and the digestion results are presented in Fig. 4.

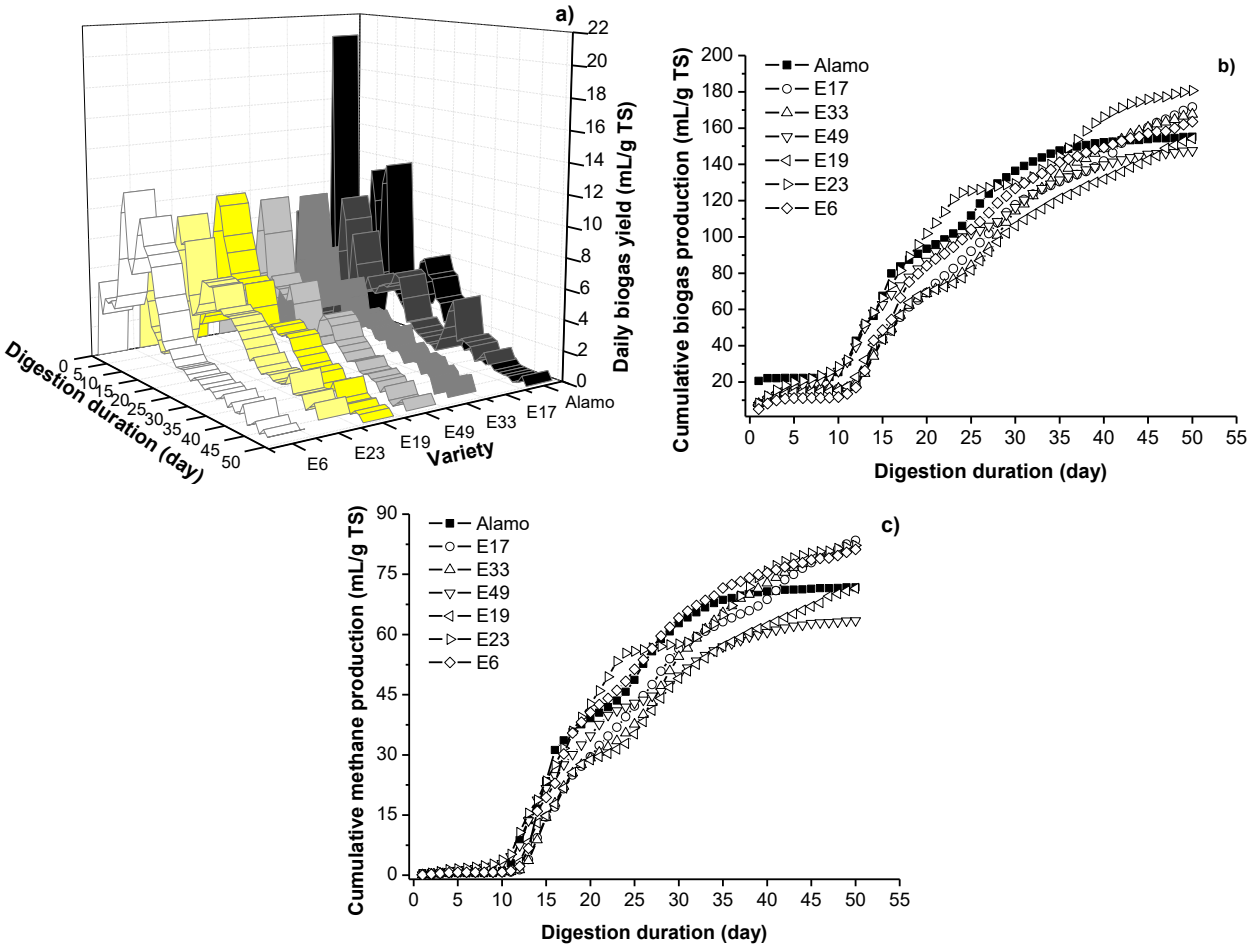

Fig. 4. Performances of anaerobic digestion and biomethane production from *Erianthus arundinaceus*. a) Daily biogas yield, b) cumulative biogas yield, and c) cumulative biomethane yield

Overall, the daily biogas production displayed three peaks, which was similar with the anaerobic digestion of lignocellulosic biomass (Wang *et al*. 2016). A rapid biogas release was observed during the first 1 to 3 days after the biomass was inoculated, and the main composition of biogas was H_2 (data not shown), which may be mainly correlated with the soluble fractions or easily degradable fractions in the biomass. When the extractives contents in biomass were linearly correlated with their corresponding H_2 yields in the first three days, the positive relationship could be observed with the correlation coefficient of 0.9089, partially proving that the rapid biogas release at the initial period was associated with the extractable fractions. The second peak of biogas release in these groups appeared at 10 to 14 days after biomass inoculation, and typically lagged compared with the lignocellulosic biomass digestion in most references, which may relate to the employed inoculum with a relatively lower activity. Similarly, the third peaks of biogas release

appeared at 15 to 18 days. Overall, there were no obvious lags or rushes on the biogas release from *E. arundinaceus* compared with switchgrass. According to the cumulative biogas production, it was 155.2 mL/g TS for the switchgrass, which was relatively lower than the reported work on anaerobic digestion of switchgrass due to the activity of employed inoculum as mentioned above (Niu *et al*. 2015; Zheng *et al*. 2015). There were four varieties of *E. arundinaceus*, including E17, E33, E23, and E6, producing more biogas than that of switchgrass (see Fig.4b). The biogas yield was promoted by 10.6%, 8.0%, 16.4%, and 5.4%, respectively. The biogas yield from the variety of E19 was almost equal to that of switchgrass, and 5.0% lower happened on the variety of E49. The cumulative methane production of these feedstocks rapidly increased from day 11 to day 13, the switchgrass and the variety of E49 achieved their corresponding maximum production after 50 days' digestion for no significant increases on the methane release from 45 days. The methane release still could be observed at the day of 50 in the varieties of E17, E33, E19, E23, and E6, in which there were 16.3%, 14.0%, 14.6%, and 13.1% promotion on the total methane yield in contrast to the switchgrass, respectively. The methane yield from E19 was almost equal to that of switchgrass. As a result, the varieties of E17, E33, E23, and E6 displayed superior performances on biomethane conversion compared with switchgrass. Overall, the methane yields from these feedstocks were lower than the reported results in references (Niu *et al*. 2015; Zheng *et al*. 2015). Therefore, pretreatment on these feedstocks and potential optimization of the anaerobic digestion should be further considered in future work.

Bioethanol and Biomethane Yields from *E. arundinaceus*

Based on the results above, the performances on bioethanol and biomethane conversion from *E. arundinaceus* were different among the six varieties and switchgrass. In practice, the actual yield of energy output from biomass was decided by the conversion performance and the annual biomass yield in the field. In the field experiments, the dry biomass yields of the investigated feedstocks were estimated, and the potential yields of bioethanol and biomethane from *E. arundinaceus* were calculated (Table 4).

Table 4. Estimations on Bioethanol and Biomethane Yield and their Energy Output from *E. arundinaceus*[#]

	Alamo	E17	E33	E49	E19	E23	E6
Biomass yield (t/ha, dry basis)	15.4	46.9	41.7	43.0	45.6	40.9	37.2
Bioethanol conversion rate (g/kg feedstock, dry basis)	141.0	116.2	144.2	146.9	149.6	130.2	137.1
Biomethane conversion rate (L/kg feedstock, dry basis)	71.8	83.5	81.8	63.5	71.5	82.2	81.2
Bioethanol yield (kg/ha)	2172	5449	6008	6308	6820	5321	5098
Biomethane yield (m^3/ha)	1105	3916	3409	2726	3258	3362	3019
Energy output of bioethanol (MJ/ha)	65900	165000	182000	191000	206900	161400	155000
Energy output of biomethane (MJ/ha)	39700	140500	122300	97800	116900	120600	108300

Note: [#] Biomass yield was obtained from actual determination in field; the bioethanol conversion rate actually was based on the SSF with 15% solid loading; biomethane conversion rate actually was based on the anaerobic digestion with 3% TS loading; yields of bioethanol and biomethane were calculated by conversion rate × biomass yield; the energy output from bioethanol or biomethane was calculated by the bioethanol or biomethane yield × the low heating value, in

which the low heating value for ethanol is 30.3 MJ/kg, and low heating value for methane is 35.9 MJ/Nm^3.

The yields of *E. arundinaceus* were significantly higher than those of switchgrass (Table 4). The E17 variety yielded the highest biomass of 46.9 t/ha annually, and the lowest yield of *E. arundinaceus* appeared with E6 (37.2 t/ha). The yields of other varieties of *E. arundinaceus* were in the range of 40.9 t/ha to 45.6 t/ha. According to the calculated results on the bioethanol and biomethane yields, E19 obtained the highest bioethanol yield of 6820 kg/ha, followed by the varieties of E49 and E33, with yields of 6308 and 6008 kg/ha, respectively. Although E17 had the highest biomass yield, the lowest bioethanol conversion had a bioethanol yield significantly lower than that of E19, E49, and E33. As for the biomethane yield, the varieties of E17 and E23, which are very easy to pretreat (as seen in the solid and cellulose recovery in Table 2), exhibited considerable biomethane yields of 3916 m^3/ha and 3362 m^3/ha, respectively, due to their relatively higher biomethane conversion and biomass yield. This result also can partially establish that the micro-structure of these two varieties was less recalcitrant compared with the other varieties. The E33 variety of achieved a considerable biomethane yield of 3409 m^3/ha. Overall, the bioethanol and biomethane yields from *E. arundinaceus* were typically higher than those of switchgrass due to the significantly higher biomass yield of *E. arundinaceus*, although the conversion rates for bioethanol and biomethane of *E. arundinaceus* were not totally superior to the switchgrass. The E17 and E19 varieties were positively recommended for biomethane and bioethanol production, respectively, and E33 can be selected as a flexible variety for bioethanol or biomethane production in practice.

Comparing the energy output, the bioethanol from 1.0 ha was typically higher than the biomethane. The biomethane yield of switchgrass in this work was approximately 34% to 40% lower than the reported references (Massé *et al*. 2010; El-Mashad 2013; Zhao *et al*. 2017) because of the relatively low activity of the employed inoculum in this work. Consequently, the energy output from biomethane conversion should be comparable and competitive to that of bioethanol production once anaerobic digestion was adjusted to normal. Additionally, bioethanol production requires pretreatment; ethanol release is reduced greatly otherwise. In contrast, the pretreatment step was not necessary for biomethane production, although it may be beneficial to increase the yield. Thus, the energy input for the pretreatment step will make biomethane production more competitive than bioethanol production. However, the net energy output and economy for the bioethanol and biomethane production from *E. arundinaceus* deserves more in-depth analysis in the future.

CONCLUSIONS

1. Based on the chemical composition, heating values, ecological adaptability, and biomass yield, *E. arundinaceus* is a potential crop for bioenergy production, mainly because of its higher biomass yield.
2. The E19 variety is recommended for bioethanol production after PHP pretreatment for its excellent bioethanol conversion rate at higher solid loading of 15% and relatively higher biomass yield.
3. The E17 variety is suggested for methane production for its highest biomass yield and biomethane conversion.

4. The E33 variety deserves attention in further work on variety breeding, cultivation, and applications for its flexibility in bioethanol and biomethane production.

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