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Title	Optimization of liquid hot water pretreatment on Hybrid Pennisetum anaerobic digestion and its effect on energy efficiency
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Publication date	2020-03-31
Original Citation	Kang, X., Zhang, Y., Lin, R., Lia, L., Zhen, F., Kong, X., Sun, Y. and Yuan, Z. (2020) 'Optimization of liquid hot water pretreatment on Hybrid Pennisetum anaerobic digestion and its effect on energy efficiency', Energy Conversion and Management, 210, 112718 (11pp). doi: 10.1016/j.enconman.2020.112718
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.enconman.2020.112718
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Download date	2026-11-06 00:56:44
Item downloaded from	https://hdl.handle.net/10468/9855

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Optimization of liquid hot water pretreatment on Hybrid *Pennisetum* anaerobic digestion and its effect on energy efficiency

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Abstract: In this study, a central composite surface response methodology was used to optimize the process variables (temperature, reaction time and liquid-to-solid ratio) in liquid hot water pretreatment for biomethane production from the anaerobic digestion of Hybrid *Pennisetum* (a typical energy crop in southern China). Temperature, reaction time and water content were varied between 160 – 200 ℃, 20 – 60 min and 60 – 90 mL respectively. The biomethane yields from pretreated material were considered as response variables to different processes conditions. Results showed untreated Hybrid *Pennisetum* had a biomethane potential of 218.6 L/kg volatile solid (VS) (corresponding to a biodegradability of 62.6%). The effect of liquid hot water pretreatment on the biomethane yield was diverse. Pretreatment severities below 4.55 resulted in increased biomethane yields. However, further increasing the severity resulted in lower biomethane yields. The optimum biomethane yield (290.6 L/kg VS) was obtained at 175 ℃ and 35 min, with the highest biodegradability of 83.2%. Energy balance showed that, under the optimal pretreatment condition, the highest theoretical energy conversion efficiency of 76.1% was achieved. If the process energy inputs are included, a process energy efficiency of 51.7% could be realized with proper heat recovery.

Keywords: Energy crops; Pretreatment; Liquid hot water; Anaerobic digestion; Response surface methodology; Energy efficiency.

1. Introduction

The transition to a sustainable and bio-based economy is crucial to reduce the dependence on fossil fuels-based energy as it is non-renewable and produces greenhouse gases such as CO_2 [1]. Bioenergy, produced from lignocellulosic biomass, is believed to play an important role in the transition progress and has attracted widespread attention [2], [3], [4].

Lignocellulosic biomass, mostly composed of cellulose, hemicellulose and lignin, including agricultural residues (such as rice straw and wheat straw), forestry residues and energy crops [5], [6], [7], [8], is a promising resource for biofuel production because it does not produce extra CO_2 as the CO_2, released in subsequent uses, was absorbed by the plant during growth [9]. Energy crops, such as switchgrass, elephant grass and *Miscanthus*, have been intensively investigated for bioenergy production due to its high dry matter yield, stress resistance (such as drought tolerance, cold resistance and heavy metals endurance) and low nutrients requirement [10]. However, a low biofuel production after biological conversion is always obtained due to the inherent recalcitrance of lignocellulosic biomass; for example, the biomethane yields previously achieved were between 20% and 60% of the theoretical yield from various substrates [9], [11], [12], [13]. Therefore, pretreatment prior to the biological conversion process is recognized as an efficient way to improve biofuel production.

Pretreatment methods can be classified into physical, chemical, biological methods and their combinations [14]. These developed methods have been considered efficient in either reducing the particle size of biomass or altering the structure of biomass, resulting in improved accessibility of cellulose; as such it improves the biodegradability of biomass [14], [15]. For example, Tsapekos et al. [16] reported the methane yield from mechanically pretreated meadow grass increased to 353 L/kg VS with an improvement of 12.3% in comparison with the methane yield from untreated samples (314 L/kg VS). With the efficient lignin removal, the methane production of alkaline pretreated sunflower stalk increased from 152 L/kg VS to 191 L/kg VS with an improvement of 26% [17]. Among all the different pretreatment methods, liquid hot water (LHW) pretreatment is an effective physicochemical method for lignocellulosic biomass by deconstructing its rigid polymer structure [18]. LHW pretreatment only uses water in the liquid state at elevated temperature as solvent without the addition of chemicals, whilst recovers the majority of pentosans from hemicellulose in the liquid fraction [19]. LHW pretreatment under 240 ℃ is highly efficient in dissolving hemicellulose with a limited effect on cellulose, which thereby exposes cellulose into the enzymes or microorganisms, and increases the biodegradability and biofuel production from the substrates [20]. With 89% removal efficiency of hemicellulose after LHW pretreatment at 175 ℃ 30 min, the methane production from AD of wheat straw increased by 63% compared to that from raw wheat straw [21]. However, as the chemical composition and structure vary in

different biomass, there is no uniform LHW pretreatment conditions for different types of lignocellulosic biomass [22], which necessitates the optimization of LHW pretreatment conditions for a specific substrate.

Hybrid *Pennisetum* (*Pennisetum americanum*×*P. purpureum*, HP), a C_4 photosynthesis perennial grass, has recently received considerable interest for bioenergy production because it can grow in marginal lands and has a high dry biomass yield with a high cellulose and hemicellulose content of up to 60% [23]. It is widely planted in south China, northeast China and the middle-lower Yangtze Plain [24], and its annual dry matter yield is estimated at 60 – 130 tons/hm^2 [25]. It has been proved that HP is a promising substrate for bioethanol and biomethane production [26], [27]. Without pretreatment, the biomethane production from the AD of HP was 208 – 249 L CH_4/kg VS, which increased to 288 – 302 L CH_4/kg VS after physical or chemical pretreatment, equivalent to an increase of 8 – 39% [23], [28], [29].

It has been well known that lignin is a barrier in the AD process for methane production from lignocellulosic biomass, and its removal can greatly improve the methane production [29]; meanwhile, the removal or dissolution of hemicellulose is also believed to have a positive impact on the methane production as the covalent interactions between lignin and hemicellulose are a hurdle for downstream utilization [30]. Yu et al. [31] reported that LHW pretreatment effectively facilitated the

enzymatic digestibility of HP by 67% after up to 98% of hemicellulose was removed. However, the study associated with the effects of LHW pretreatment on the AD of HP is not investigated. In an effort to understand the correlation between enzymatic digestibility and anaerobic biodegradability (ABD) of lignocellulosic biomass, Jung et al. [32] reported that there was an unreliable linear relationship (R^2 = 0.70) between enzymatic digestibility and ABD, which was also observed by Frydendal et al. [33]. Moreover, Wang et al. [34] found higher pretreatment temperature (200 ℃), even with almost complete removal of hemicellulose, issued in decreased methane production from rice straw. Therefore, in order to exploit the optimum biomethane production from HP, it is essential to investigate the optimal LHW pretreatment conditions for the AD of HP; as the LHW pretreatment is an energy intensive method, the energy conversion efficiency and process energy efficiency of the integrated system of LHW pretreatment and anaerobic digestion need to be evaluated for its practical application. Herein, the objective of this study is to (1) evaluate the effect of LHW pretreatment on the chemical composition and structure of HP, (2) optimize the LHW pretreatment conditions to achieve the optimal methane production using response surface methodology and (3) assess the energy efficiency of the integrated LHW pretreatment and AD system by computing energy balance and energy process efficiency.

2. Materials and methods

2.1 Material and inoculum

HP was harvested from Zengcheng District, Guangdong Province, China, in June

2016. The raw and fresh material was first cut into 2-3 cm, and then smashed for 1 min in a pulverizer (XB-HP, Xiaobao, China). The smashed samples were dried at 85 ℃ for 3 days, and the dried samples were sieved with sieve mesh. The granulometric fraction (20~80 mesh) was stored in a desiccator at room temperature before use.

The characteristics of raw material and inoculum are shown in Table 1. Inoculum was derived from a mesophilic continuously stirred tank reactor fed with cellulose and grass. Before use, the inoculum was degassed for 7 days.

2.2 Liquid hot water pretreatment

Liquid hot water (LHW) pretreatment was conducted in a 100 mL Teflon-lined stainless autoclave reactor (Dalian Tongda Science and Technology Development Co., Ltd. China), which can stand a temperature of up to 300 ℃and a pressure of 22 MPa. During the LHW pretreatment, process parameters including temperature, time and liquid-to-solid (L/S) ratio were investigated. The three parameters used in this study were based on the research from Yu et al. [31] who reported that the optimal pretreatment condition was 180 °C 40 min for HP, in which the enzymatic digestibility was improved by 66.9% and pentose recovery was improved to 98.1%, respectively. Therefore, the pretreatment temperature and reaction time used as the central points for surface response design in this study was chose to be 180 °C and 40 min, respectively. In detail, 3 g VS samples were firstly added to the reactor loaded

144 with 60-90 mL of deionized water according to different L/S ratios (v/vwt (volatile
145 weight)) of 20:1, 25:1 and 30:1, then the mixture was sustained at 160, 180 and
146 200 °C with different reaction time of 20, 40 and 60 min. Each experiment was
147 performed in quadruplicate. After pretreatment, the pH value for three samples of the
148 quadruplicates was neutralized using 1 M NaOH for the subsequent AD process. One
149 of the quadruplicates was filtered and washed with same amount of the distilled water
150 in the reactor. The liquid fraction was stored at 4 °C for further analysis; the solid
151 residues were used for solid recover and chemical composition analysis.
152
153 **2.3 Biochemical methane potential assay**
154 The effects of LHW pretreatment on the conversion efficiency and kinetic properties
155 of HP were assessed using automatic biomethane potential test system (Bioprocess
156 Control Sweden AB, AMPTS II) to conduct biochemical methane potential (BMP)
157 assays. The untreated and LHW pretreated samples, in triplicates, were separately
158 tested for each set of experimental conditions. In detail, the BMP were set as
159 following: inocula (400 mL) and the material (both solid and liquid fraction) were
160 added at the ratio of about 1 based on VS as reported previously [23], [35]. Deionized
161 water was added to reach the working volume of 500 mL. For each run, the methane
162 yield originating from the inoculum was measured using one blank including 100 mL
163 deionized water and 400 mL inoculum. All experiments were performed at 37 ± 0.5 ℃
164 in an electric-heated thermostatic water bath and lasted for 35 days.

2.4 Analytical methods

The TS, VS and ash contents were evaluated based on the method as previously descripted by Li et al. [36]. The method of acquiring scanning electronic microscope (SEM) image was described before [28]. The chemical composition of untreated and LHW pretreated samples was analyzed using two-step sulfuric acid hydrolysis method according to Sluiter et al. [37]. The liquid fraction, collected from the washing process after pretreatment, was used to measure the quantification of sugars (including monomeric sugars, oligomeric sugars and total sugars), organic acids and hydroxymethyl furfural (HMF) in the pretreatment hydrolysates. The measurement was conducted by HPLC (Waters e2695) using a Shodex sugar SH-1011 column coupled with refractive index detector and UV detector. The column temperature was 50 ℃with a mobile phase of 0.005M H_2SO_4 at a flow rate of 0.5 ml per minute. The quantification of total sugars was determined after a secondary hydrolysis with 4% (w/w) sulfuric acid at 121 °C for 60 min as previously reported [31].

2.5 Experimental design and analysis

As aforementioned, the pretreatment experiment was designed using a central composite response surface methodology (RSM) with 3 central point using Design Expert 11 based on the results from Yu et al. [31]. Three independent process variables; temperature, reaction time and L/S ratio were varied between 160 – 200 °C, 20 – 60 min and 20:1 – 30:1 respectively. All other process parameters such as heating

rate, the rate of stirring and the mass concentration were kept constant for each experiment. This resulted in 17 experimental runs as shown in Table 2. Methane yield was used as response variables to evaluate the effect of the process parameters in the LHW pretreatment process. The severity factor ($logR_0$), a measure of the pretreatment intensity in hydrothermal process, which is a function of temperature (T) and reaction time (t) was calculated using Eq. (1) (Ferreira et al., 2013).

$$logR_0 = log(t \times exp((T-100)/14.75)) \quad (1)$$

The data from the BMP assays of the pretreated samples were used to fit the quadratic equations generated by Design Expert 11 (Eqs. (2)).

$$Y = \zeta_0 + \zeta_1 A + \zeta_2 B + \zeta_3 C + \zeta_{11} A^2 + \zeta_{22} B^2 + \zeta_{33} C^2 + \zeta_{12} AB + \zeta_{23} BC + \zeta_{13} AC \quad (2)$$

where Y represents the response variable for methane yield (mL/g VS); ζ_0, ζ_1, ζ_2, ζ_3 mean coefficients of linear expressions; ζ_{11}, ζ_{22}, ζ_{33} are the quadratic coefficients and ζ_{12}, ζ_{23}, ζ_{13} are interaction coefficients; A, B and C are the independent variables, namely, pretreatment temperature, reaction time and L/S ratio. Then, the predicted optimal values were confirmed by the verification tests using the adjusted values of the three variables.

The process dynamics, including decay constant ($days^{-1}$) and maximum yield (Y_{max}) on the basis of BMP results from untreated and optimized LHW pretreated substrates, were assessed by a first order differential equation (Eq. (3)). T_{50} (half time) was

defined as the time needed to achieve 50% of the maximum cumulative biomethane production. The biodegradability index (BI) was the ratio of BMP yield to the theoretical methane yield.

$$Y(t) = Y_m \times (1 - e^{(-kt)}) \quad (3)$$

Where t is the digestion time (days); k means the decay constant, which presents the rate of degradability of the substrate; $Y(t)$ is the cumulative methane yield (mL CH_4/g VS) at time t (days); Y_m refers to the maximum methane yield (mL CH_4/g VS).

2.6 Energy balance

2.6.1 Process energy input

The process energy inputs comprise the energy use in LHW pretreatment and AD. The total energy needed (E_{in}; kJ/g fresh weight) can be reckoned using Eq. (4) modified from Lin et al. [38]:

$$E_{in} = E_{LHW} + E_{AD} \quad (4)$$

Where E_{LHW} is the energy input for LHW pretreatment and E_{AD} is the energy input for AD. The energy input for each process can be estimated by Eq. (5):

$$E_{LHW}/E_{AD} = (V_{reac}\rho_w Cp_w + m_{s,fresh}Cp_{s,fresh}) \times (T_{reac} - T_{room}) \quad (5)$$

Where the liquid fraction in the reactor (V_{reac}) is presumed as water with a heat capacity (Cp_w) of 4.18 kJ/kg/K. The LHW pretreatment temperature (T_{reac}) is chosen to be 160, 175, 180 and 200 °C in which the V_{reac} was the same to guarantee the temperature was the only independent variable; the room temperature (T_{room}) is assumed as 25 °C. The temperature of AD is 37 °C according to the experimental

set-up. $m_{s,fresh}$ represents the fresh weight of HP added into the reactor, and $Cp_{s,fresh}$ is the corresponding heat capacity based on the fresh mass. The heat capacity (kJ/kg/K) of HP is calculated as per Dupont et al. [39] using Eq. (6)

$$Cp_{biomass} = 5.340Tp - 299 \quad (6)$$

Where Tp is the specific temperature for calculating the heat capacity of biomass. It is assumed that the heat capacity of HP is not changed with pretreatment or AD and mass loss between the different process is neglectable.

2.6.2 Energy conversion efficiency and process energy efficiency

The process energy output refers to the energy yield of methane produced from AD as per Eq. (7):

$$E_{CH4} = V_{CH4,VS} \times HV_{CH4} \quad (7)$$

$V_{CH4,VS}$ (mL/g VS) is the volumetric yield of methane per unit VS substrate added. The energy conversion efficiency (β_{ece}) is defined as the ratio of the heating value (HV) in methane to the total heating value of the added substrate [40].

$$\beta_{ece} = E_{CH4}/ E_{HP} \times 100\% \quad (8)$$

The energy value of HP is calculated based on the following formula (Eq. (9)) [41]:

$$E_{HP} = 337C + 1419(H - 1/8O) + 23.26N \quad (9)$$

In which C, H, O and N refer to the respective weight percentage of each element in total VS. Accordingly, given the energy input associated with LHW pretreatment and AD, the overall process efficiency can be represented on the basis of Eq. (10):

$$\beta_{pee} = (E_{CH4} + E_{recov}) / (E_{LHW} + E_{AD} + E_{HP}) \times 100\% \quad (10)$$

where E_{recov} is the probable heat recovery from LHW pretreatment (this would be 0 as per the experimental set-up). The heat recovery was calculated considering cooling down the pretreatment hydrolysates from process temperatures to 50 °C [38] with an assumed efficiency of 90% of a heat exchanger [42]. No heat recovery is obtained as per the batch experimental set-up. However, as the LHW pretreatment is operated at elevated temperatures, cooling down of the pretreated HP hydrolysates would provide a promising opportunity for heat recovery with its subsequent use in providing heat for AD.

2.7 Statistical analysis

The data of chemical compositions and specific methane yields of untreated and LHW pretreated samples were statistically analyzed utilizing SPSS 22.0 software with one-way analysis of variance (ANOVA) at a probability level of 0.05.

3. Results and discussion

3.1 Effects of LHW pretreatment on the chemical compositions of Hybrid *Pennisetum*

3.1.1 Effect of LHW pretreatment on chemical composition of solid fraction

Solid recovery is an important parameter of consideration regarding to AD; higher temperatures in LHW pretreatment would result in an increase of lignin content in the recovered solid fraction which may lead to a lower overall methane yield [43]. Table 2

displays the solid recovery and chemical composition of untreated and LHW pretreated HP. Overall, the solid recovery decreased as the temperature and reaction time increased mainly due to the removal of xylan; while the L/S ratio had insignificant effects on the solid recovery, which agreed with Perez et al. [44] who observed that the solid recovery of LHW pretreated wheat straw decreased with elevated temperature and reaction time. For example, with the same pretreatment time of 60 min, the solid recovery of HP reduced from 69.2% to 56.6% and further decreased to 50.1% as the temperature increased from 160 ℃ to 180 ℃ and further increased to 200 ℃; meanwhile, with the same pretreatment temperature such as 180 ℃, the solid recovery was reduced from 71.8% to 56.6% as the reaction time increased from 20 min to 60 min. However, it was found that there were no significant effects of L/S ratio on the solid recovery when the temperature and reaction time were set as the same. Li et al. [45] reported that the solid recovery of HP was 65.9% after LHW pretreatment at 180 ℃ for 60 min with a L/S ratio of 10:1, and attributed the solid loss to the removal of hemicellulose as it accounted for 67.7% of the total mass loss.

LHW pretreatment is efficient in dissolving hemicellulose while producing less inhibitory compounds compared to other chemical pretreatments [22]. As shown in Fig. 1, the hydrolysis efficiency of xylan was relatively low in three low-intensity tests ($\log R_0 < 3.4$), suggesting that LHW pretreatment at low temperatures could not

294 significantly remove hemicellulose from HP (data can be found in the supplementary
295 material). Yu et al. [26] also reported that the removal efficiency of hemicellulose was
296 low (16%) under the condition of 160 ℃ when applying LHW pretreatment to energy
297 sorghums. As the severity increased to medium conditions ($logR_0$ < 4.2), the
298 degradation of xylan greatly increased to 58.0%. The removal efficiency of xylan
299 increased to almost 100 % when the temperature increased to 200 ℃ with the severity
300 factor over 4.2. This was because that under high temperature, the autoionization of
301 H_2O produced hydronium ions (H_3O^+), which acted as an acid catalyst breaking down
302 the covalent bond of lignin and carbohydrates, leading to the dissolution of
303 hemicellulose [46]. At the same temperature of 200 ℃, the degradation efficacy of
304 hemicellulose in *Tamarix ramosissima* was 89% for 3 h [47]; while the complete
305 removal of hemicellulose in beach wood was observed at 220 ℃ for 15 min with a
306 severity factor of 4.69 [48]; these results indicated that compared to woody biomass,
307 HP was prone to hydrolysis under mild hydrothermal condition, which could reduce
308 the energy input during the pretreatment.
309
310 During LHW pretreatment process, lignin was also degraded. Although there was an
311 increase in lignin content in the residues at higher temperatures due to the decrease of
312 xylan, mass balance analysis revealed that the removal of lignin was about 40% for all
313 the tests (Fig. 1) (data can be found in the supplementary material). Lignin removal
314 efficiency of other lignocellulosic biomass was also limited when employing LHW

315 pretreatment on wheat straw [34] and poplar [20], respectively. It has been well
316 elucidated that the formation of pseudo-lignin took place under severe conditions
317 (high temperature and long reaction time) [49], which may explain the high lignin
318 content (26-29%) in the residues after LHW pretreatment at 200 ℃. Along with the
319 xylan and lignin degraded during the pretreatment, the glucan content in the residues
320 (Table 2) relatively increased to 34.1 – 65.2% ($p<0.05$), with a recovery of up to
321 100%, indicating that in comparison to xylan and lignin, the impact of LHW
322 pretreatment used in this study on the cellulose was minimal due to its thermal
323 stability and unique structure [50].
324
325 Further effort was conducted to investigate the relationship between chemical
326 composition variations and severity factor. As presented in Fig 1, there was a positive
327 relationship between severity factor and the removal of xylan, and glucan content in
328 the residues with the correlation coefficient (R^2) of 0.86 and 0.83, respectively.
329 Namely, along with the severity factor increase, the glucan content in the residues and
330 the removal efficiency of xylan increased. This agreed with Yang et al. [51] who
331 observed that up to 90% of xylan in corn stover was removed when the severity was
332 higher than 4.3. However, lignin removal appeared to bear no relation to severity
333 factor with R^2 of 0.07. Ko et al. [52] obtained similar results when investigating the
334 effects of severity factor on the lignin properties of LHW pretreated hardwood; these
335 can be attributed to the condensation reactions of lignin with other degradation

productions during the pretreatment process [53]. The mechanism of hemicellulose removal during LHW pretreatment has been well elucidated in a previous review [54]; the authors demonstrated that besides the hemicellulose removal, organic acids such as acetic acid and formic acid are generated. These organic acids, the desirable intermediates for AD, can be effortlessly converted into biomethane by methanogenic archaea [55]; it was also reported that these organic acids in turn facilitated the pretreatment [56].

3.1.2 Effect of LHW pretreatment on chemical composition of liquid fraction

Above analysis illustrated that after LHW pretreatment, the main compositions of the solid residue were cellulose and lignin, while hemicellulose-derived degradation products existed in the liquid fraction. Table 3 presents the chemical components of the liquid fraction. Overall, the liquid fraction contained oligosaccahrides, monosaccharides (glucose, xylose and arabiose) and organic acids (acetic acid, glucuronic acid, glycolic acid, formic acid and levulinic acid), the majority of which can be derived from the hemicellulose composition in HP. Given the respective content of xylan and araban in the raw material is 20.8% and 2.7%, it can be concluded that the structure of the hemicellulose from HP is an arabinoxylan; moreover, considering the chemical composition in the liquid fraction, the arabinoxylan could be mainly in the form of *O*-acetyl-4-*O*-methyl-glucuronoarabinoxylan, which is the typical structure of

hemicellulose in Gramineous biomass such as Vetiver grass [57] and sweet sorghum [58]. Fig. 2 exhibits the structure of *O*-acetyl-4-*O*-methyl-glucuronoarabinoxylan and its proposed decomposition pathway in LHW pretreatment; it consists of β-1,4-D-xylan backbone where *O*-2 is substituted by α-D-glucuronic acid residue, and *O*-3 is substituted by α-L-arabinose residue and acetyl group [57], [58]. The inhibitory compounds such as furfural and hydroxymethyylfurfural were not detected in all the experiments. The total glucose in the liquid fraction showed a tendency of decrease from 2.13 g/L to 0.84 g/L as the temperature increased from 160 to 200 ℃, indicating the further degradation of glucose under severe conditions. However, the concentration of arabinose increased from 0.35 g/L to 1.54 g/L with the temperature increased from 160 to 180 ℃, indicating that the hydrolysis effacy of xylan was strenghthened by increased temperature due to the formation of hydronium ions (H_3O^+). It was well elucidated that hemicellulose removal efficiency increased with elevated temperature and longer retention time, which was mostly solubilized as oligomers during LHW pretreatment [51], [59]. The concentration of xylooliglosaccharides and xylose is presented in Fig.3. At 6 low-intensity tests ($logR_0 < 4.0$), the concentration of total xylose (xylose oligomer and xylose) was low (0.75~1.61 g/L), confirming the limitation of LHW pretreatment on dissolving hemicellulose at low temperature and short reaction time. The concentration of total xylose at medium-intensity ($4.0 < logR_0 < 4.5$) was higher than that in low-intensity tests, which was 4.02–6.30 g/L. While under the harsh conditions ($logR_0 > 4.5$), the

concentration of total xylose decreased to 0.65–2.95 g/L due to the further degradation of xylose to the organic acids such as formic acid, which can be confirmed by the subsequent analysis of organic acids. Phuttaro et al. [4] observed similar results when treating Napier grass by LHW pretreatment. Li et al. [45] disclosed that the degree of polymerization of xylooligosaccharides in LHW pretreatment hydrolysate was less than 6, pointing out that under the same LHW pretreatment condition, the hydrolysis efficiency of hemicellulose in HP was higher than that in switchgrass and *Miscanthus*.

At 160 ℃, the main organic acids were acetic acid (0.23–0.32 g/L) and glucuronic acid (0.21–0.28 g/L), while acetic acid was the main organic acid at 180 ℃ (Table 2). Acetic acid was produced from the acetyl group in *O*-acetyl-4-*O*-methyl-glucuronoarabinoxylan in LHW pretreatment [46] and it can be concluded from aforementioned results that under 180 ℃, the hemicellulose in HP was hydrolyzed to oligoxylose, arabinose, glucuronic acid and acetic acid following the initial hydrolysis pathway in Fig. 2. As the temperature increased to 200 ℃, the main organic acids were acetic acid (0.67–1.33 g/L) and glycolic acid (0.42–1.07 g/L). Glycolic acid was derived from glucuronic acid through the subsequent hydrolysis pathway as shown in Fig. 2 (the degradation mechanism of glucuronic acid can be found in supplementary material). It can be concluded that as the temperature increased, the autoionization process was enhanced and thereby intensified the hydrolysis of hemicellulose from HP, which increased the concentration of acetic acid

399 derived from the acetyl group; the resulting acetic acid in turn facilitated the
400 hydrolysis process [56], leading to the complete removal of hemicellulose from HP
401 under 200 °C. Meanwhile, the further degradation of arabinose and glucuronic acid
402 led to the generation of formic acid and levulinic acid [34], [56]. The formation of
403 monosaccharides and organic acids confirmed the deconstruction of the substrates,
404 suggesting a positive effect on the AD process as the hydrolysis is the limitation step
405 in the AD process of biomass [54].
406
407 **3.2 Optimization of anaerobic digestion of liquid hot water pretreated Hybrid**
408 ***Pennisetum***
409 The deconstruction of HP and formation of intermediates may facilitate the AD
410 performance of HP (SEM image can be found in supplementary document). It has
411 been reported that LHW pretreatment significantly increased methane production
412 from biomass, such as wheat straw [60], Napier grass [4] and microalgae [61].
413 However, some researchers reported that higher temperature during LHW
414 pretreatment could inhibit the AD of biomass, leading to a lower methane yield [34],
415 [62]. Therefore, in order to ascertain the optimal LHW pretreatment condition for the
416 AD of HP, the RSM was used to optimize the methane production from the AD of
417 LHW pretreated HP.
418

3.2.1 Response surface model analysis

Table 4 shows the analysis of variance for the regression quadratic model equation of central composite design. The Model F-value of 4.49 implied the model was significant. There was only a chance of 3.01% that a "Model F-Value" this large could occur due to noise. Values of "Prob>F" less than 0.05 indicated model terms were significant. In this case, A and A^2 were significant model terms. The "Lack of Fit F-value" of 25.26 suggested the lack of fit was significant. There was only a 0.46% chance that a "Lack of Fit F-value" this large could occur due to noise. The determination coefficient (R^2) was used to measure the quality of the model equation fitting and to decide the amount of variation explained by the model. In the observed response values, the correlation coefficient, $R^2 = 0.8525$, demonstrated that the sample variation of 85.25% in the methane yield response could be explained by the model. A good statistical model should have R^2 value in the range of 0.75–1 [63]. Adequation precision measured the signal to noise ratio and a ratio greater than 4 was preferable. The ratio of 7.184 indicated an adequate signal, meaning that this model can be used to navigate the design space. The percentage of coefficient of variation (CV %) is a measure of residual variation of the data relative to the size of the mean. Usually, the higher the CV value, the lower the reliability of experiment. Hence, a lower value of CV (6.43%) exhibited a greater reliability of the experiment. The predicted residual sum of squares (PRESS) is a measure of how well the model fitted each point in the design. The smaller the PRESS statistics, the better the fitness between the model and

the data points. Here the value of PRESS was found to be 31021.74 meaning the model well fitted the data points. Multinomial regression analysis was carried out on the experimental data, and the quadratic regression equation was shown as following:

$$Y = 288.98-21.39A-9.36B+3.98C-19.50AB+2.42AC-5.17BC-32.56A^2-11.01B^2-7.79C^2$$

The response surface of methane yield and the corresponding contour plots were displayed as Fig. 4. When the ratio of L/S was 25:1 (75 mL water: 3 g VS sample), the combined effect of temperature and time on methane yields was shown in Fig. 4(a–b). From Fig. 4(a), the methane yields gradually increased with the increase of temperature and time while decreased as the temperature increased to 200 ℃. The 3-D surface was steep, and the contour line tended to an ellipse, which all indicated that there was a significant effect of temperature on the methane production. From Fig. 4(b), when the temperature and time were controlled at the range of 168 – 190 ℃ and 35 – 50 min, respectively, the methane yield would be higher than 280 mL/g VS. The maximum methane yield could be acquired within these range of pretreatment temperature and time.

When the pretreatment time was set at 40 min, the combined effect of temperature and L/S ratio on the methane yields was displayed at Fig. 4(c–d). From Fig. 4(c), the methane yield decreased at the temperature of 180 – 200 ℃ while no significant effect

of L/S ratio on the methane yields was observed. In addition, the response surface was relatively steep, and the contour line presented an ellipsis shape, which both demonstrated that the interaction between the two factors was remarkable. As depicted in Fig. 4(d), the methane yield was not lower than 280 mL/g VS between the temperature of 165 – 180 ℃ and water of 70 – 85 mL.

When the temperature was 180 ℃, the combined effect of pretreatment time and L/S ratio on the methane yields was displayed at Fig. 4(e-f). From Fig. 4(e), the methane yield decreased at the pretreatment time of 35 – 60 min. However, though the contour line presented an ellipsis shape, the response surface was relatively flat, which demonstrated that the interaction between the two factors was not notable. As depicted in Fig. 4(f), the methane yield was not lower than 290 mL/g VS between the pretreatment time of 20–45 min and L/S of 23:1–30:1.

The three kinds of process parameters as the key factors and the highest methane yield as the optimization objective was optimized by the RSM respond surface. According to the investigation, the predicted highest of methane yield could be 293.4 mL/g VS at the optimal condition of temperature of 175.5 ℃, reaction time of 34 min, and L/S ratio of 26.6:1 (corresponding to the water content of 79.8 mL).

3.2.2 Confirmatory experiments and its kinetic study

Triplicate experiments were carried out under the adjusted best pretreatment conditions (175 ℃, 35 min and water of 75 mL) to verify the results predicted by the regression model. The obtained methane yield was 290.6 mL/g VS with 32.9% increase compared to that of 218.6 mL/g VS from the untreated HP (Fig .5b). The fine agreement between the actual and predicted values (0.95% deviation error) identified the adequacy and effectiveness of the response model to predict the methane yield from HP. Other studies also proved that RSM was a powerful tool to optimize various pretreatment conditions for biomethane/biogas production from different biomass such as rice straw [63], wheat straw [64] and fruit waste [65]. Previous studies suggested that there was no linear relationship between severity factor and methane yield from lignocellulosic biomass [33], which agreed with the results in this study. Furthermore, an inverse relationship between temperature and biomethane production was noticed during LHW pretreatment. For instance, the biomethane production from wheat straw decreased by 30% after LHW pretreatment at 210 ℃; while the optimum biomethane production was achieved after LHW pretreatment at 180 ℃ [34]. The highest biogas production of 615 mL/g VS from LHW pretreated wheat straw was achieved at 180 ℃ [60]. In another study, Shang et al. [21] achieved the highest methane production of 202 mL/g VS from wheat straw at 175 ℃ with 30 min, an increase of 63% compared to that from the untreated samples. These results proved that the LHW pretreatment used in this study was beneficial for methane production.

In addition, it was found that the AD efficiency of *Miscanthus* was improved after LHW pretreatment as the retention time of AD was decreased by 50% [10]. Kamusoko et al. [66] compared the efficacy of physical, chemical, biological and combined pretreatment methods in enhancing biomethane production from crop residues, and summarized that physical pretreatment method such as LHW pretreatment was the most effective and fastest in terms of the rate of hydrolysis during pretreatment. This further led to a shorter retention time of AD than that without pretreatment. Therefore, it was necessary to understand the effect of LHW pretreatment with optimal condition on the AD performance of HP.

Fig. 5 and Table 4 display the parameters such as the kinetic decay, T_{50} and maximum yield for understanding the process dynamics. Cellulose was used as a control to test the activity of the inocula; the average methane production from cellulose was 324.9 mL/g VS-added, indicating that the inocula were in a good condition [67]. The first order equation showed very high correlation as values of R^2 were 0.992 and 0.994 for LHW pretreated and untreated HP, respectively. Both untreated and LHW pretreated HP showed a good kinetic decay (k value was 0.23 and 0.24, respectively). The highest daily methane yield was increased from 45.55 mL/g VS/d to 55.23 mL/g VS/d at day 1 after LHW pretreatment with an increase of 21% (Fig.5 (a)). However, the time for achieving the 50% of cumulative methane yield was not profoundly shortened (from 3.01 days to 2.84 days after LHW pretreatment) (Table 4). This can

523 be attributed to the relatively good degradability of the untreated sample as its
524 biodegradability was 62.6% which was higher than that from previous studies [29],
525 [68]. This can be further explained by the results of inoculum accumulation as the
526 inoculum used in this study was fed with grass for a long time. Gu et al. [69] reported
527 that the inoculum has a great impact on the AD of biomass, and the authors suggested
528 it is essential to conduct the adaptation step before AD assay to achieve the maximum
529 methane production from the substrates.
530
531 **3.3 Energy and mass balance of Hybrid *Pennisetum* in anaerobic digestion**
532 Fig. 6 shows the results of both energy conversion efficiency and process energy
533 efficiencies under three different scenarios. These scenarios were: (1) process energy
534 efficiency under each experimental condition; (2) process energy efficiency with
535 potential heat recovery; and (3) process energy efficiency with reduced water usage
536 for LHW pretreatment by 30%. The energy conversion efficiency at all conditions
537 exhibited a higher value compared to the energy process efficiency, which resulted
538 from the exclusion of the energy input from LHW pretreatment and AD in the
539 calculation of energy conversion efficiency. The total energy conversion efficiency of
540 untreated HP was calculated as 57.2%, which was comparable with that of seaweed
541 (59.6%) [38]. The LHW pretreatment at 175 °C (optimal condition) led to the
542 maximum energy conversion efficiency of 76.1%, which again confirms the
543 sufficiency of the model. Mass balance (displayed in Fig. 7) showed that the recovery

544 of glucan, xylan and araban in the liquid hydrolysate after pretreatment at optimal
545 condition was 73.7%, 72.1% and 66.7%, respectively; meanwhile the respective
546 removal of lignin and araban was 36.2% and 100%. It was noted that not all the
547 energy of HP was converted into methane with 23.9 - 42.8% of the total energy
548 unexploited. These can be explained by following reasons: (1) the biodegradability of
549 HP was not 100%; (2) a portion of energy was consumed for microbial growth and
550 reproduction [38]; (3) lignin, also composed of C, H and O, cannot be converted into
551 methane in AD; as such the calculated heating value is higher than the actual
552 exploitable energy value [70], [71], [72].
553
554 Considering the heat energy input for LHW pretreatment and AD, the highest process
555 energy efficiency of 28.6% was obtained for untreated HP. Unlike the energy
556 conversion efficiency which peaked at 175 °C, the process efficiency tended to
557 decrease with increased temperature, due to a higher demand for external heat. This
558 result indicated that the improved methane yield was not enough to surpass the added
559 heat required to maintain the temperature of pretreatment. Even with the water use
560 reduced by as much as 30%, the process energy balance showed insignificant
561 improvement. However, with the assumption that the potential heat was recovered
562 from the hydrolysates, a significant benefit in process energy efficiency was obtained.
563 A new peak of process energy efficiency was achieved at 180 °C of 52.1%, which,
564 however, was not significantly higher than that at 175 °C of 51.7%. Hence, the

recovery of waste heat is of importance, whereby the simplest use is to sustain the heat for AD. Upon further system optimization and integration, the reported combined system may provide a new approach for HP processing and valorization.

4. Conclusion

Liquid hot water pretreatment can not only alter the structure of Hybrid *Pennisetum*, but also improve its anaerobic digestion performance under certain condition. LHW pretreatment was proven to be efficient in dissolving hemicellulose, which resulted in up to 100% of xylan removal of Hybrid *Pennisetum*. The optimal LHW pretreatment condition (175 °C, 35 min) led to 32.9% increase of methane production compared with the untreated sample. Kinetic study showed that LHW pretreated Hybrid *Pennisetum* led to a relatively short retention time in AD to achieve the 50% of total methane production. Energy balance showed that the highest energy conversion efficiency of 76.1% was achieved at the optimal pretreatment condition. The maximum process energy efficiency of 28.6% was obtained from the untreated substrate considering the energy input for pretreatment and anaerobic digestion. However, it can be significantly increased to 51.7% for the optimal pretreatment condition with heat recovery. This provides fundamental information for further system optimization and enables a possibility for energy crops utilization in an integrated thermochemical and biological process.

Acknowledgment

This work was supported financially by the National Natural Science Foundation of China [grant number 51776208]; the Strategic Priority Research Program of Chinese Academy of Sciences [grand number XDA21050400]; the Science and Technology Planning Project of Guangdong Province [grant number 2017B020238005]; the Science and Technology Program of Guangzhou [grant number 201803030007], and Agricultural Technology Research and Development Project of Guangdong Province [grant number 2018LM2178]. This work also received the support from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant (No. 797259). Dr. Yi Zhang gratefully acknowledged the "China and Germany Postdoctoral Exchange Program" (Grant No. ZD2018025) funded by the Office of the China Postdoctoral Council and Helmholtz Association.

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Table and figure captions:

Table 1. Compositional characteristics of Hybrid Pennisetum and inoculum.

Table 2. Chemical composition of untreated and liquid hot water pretreated hybrid *Pennisetum*.

Table 3. Chemical composition of liquid fraction from liquid hot water pretreated hybrid *Pennisetum*.

Table 4. The variance for the regression quadratic model equation of central composite design.

Table 5. The process dynamics of untreated and optimized liquid hot water pretreated hybrid *Pennisetum*.

Figure. 1. The correlation between severity factor and chemical composition of hybrid *Pennisetum*.

Figure.2 Proposed pathway for O-acetyl-4-O-methyl-glucuronoarabinoxylan decomposition in liquid hot water pretreatment.

Figure. 3. Concentration of xylose monomer and xylose oligomar in the liquid fraction after liquid hot water pretreatment.

Figure. 4. Response surface analysis of methane yield from the anaerobic digestion of liquid hot water pretreated Hybrid *Pennisetum* (R1: methane production; A, B and C: temperature, reaction time and water use).

Figure. 5. Daily (a) and cumulative (b) methane yield of untreated and optimal liquid hot water (LHW) treated hybrid *Pennisetum*. Cellulose was used as control group to test the activity of the inocula.

Figure. 6. Energy conversion efficiency (β_{ece}) and process energy efficiency (β_{pee}) of the anaerobic digestion of hybrid *Pennisetum*. $\beta_{p,HR}$: Considering heat recovery from cooling down of pretreated hydrolysates. $\beta_{p,LW}$: Considering 30% less water use than that in hydrothermal pretreatment without heat recovery.

Figure. 7. Overall mass balance flow diagram of Hybrid Pennisetum at optimal liquid hot water pretreatment condition and anaerobic digestion.

840 Table 1. Compositional characteristics of Hybrid *Pennisetum* and inoculum.

	Hybrid *Pennisetum*	Inoculum
Proximate analysis (wt %)		
TS	94.6 ± 0.3	2.0 ± 0.3
VS	84.9 ± 0.3	0.8 ± 0.1
VS/TS	89.7	40
Ash	9.5 ± 0.2	1.2 ± 0.2
pH value	/	8.01
Ultimate analysis (% VS)		
Carbon	40.02 ± 0.04	/
Hydrogen	5.89 ± 0.03	/
Oxygen	53.41 ± 0.04	/
Nitrogen	0.69 ± 0.04	/
C/N mass ratio	58	/
Biological analysis (% TS)		
Glucan	30.3 ± 0.2	/
Xylan	20.8 ± 0.2	/
Araban	2.7 ± 0.1	/
Lignin	24.7 ± 0.5	/
Energy value (kJ/g VS)	13.7	/
Theoretical biomethane yield (mL/g VS)	349.1	/

841

842 Table 2. Chemical composition of untreated and liquid hot water pretreated Hybrid *Pennisetum*.

Experiment No.	Temperature (℃)	Time (min)	Liquid-to-substrate ratio	Severity factor (log R_0)	Solid recovery (%)	Glucan (%)	Xylan (%)	Araban (%)	Lignin (%)
5	160	20	25:1	3.07	73.7	34.49 ± 0.64^{b}	22.92 ± 0.40^{e}	0.00	19.67 ± 0.47^{a}
16	160	40	20:1	3.37	74.9	34.20 ± 2.95^{b}	21.71 ± 1.96^{de}	0.00	20.33 ± 2.36^{a}
15	160	40	30:1	3.37	72.9	36.68 ± 0.70^{bc}	24.80 ± 2.26^{f}	1.62 ± 2.29	20.00 ± 0.00^{a}
11	160	60	25:1	3.55	69.2	34.07 ± 2.88^{b}	21.13 ± 1.73^{de}	0.00	20.67 ± 0.00^{a}
14	180	20	20:1	3.66	71.8	38.72 ± 1.07^{c}	22.57 ± 0.42^{de}	0.00	23.00 ± 0.47^{abc}
7	180	20	30:1	3.66	72.6	36.85 ± 0.45^{bc}	22.68 ± 0.42^{de}	0.00	20.67 ± 0.00^{abc}
4	180	40	25:1	3.96	67.5	44.26 ± 0.12^{d}	17.85 ± 0.24^{c}	0.00	22.00 ± 0.94^{ab}
6	180	40	25:1	3.96	68.3	44.81 ± 0.89^{d}	18.83 ± 0.10^{c}	0.00	22.33 ± 0.47^{abc}
9	180	40	25:1	3.96	58.4	44.08 ± 0.10^{d}	18.18 ± 0.06^{c}	0.00	22.33 ± 0.47^{abc}
12	180	40	25:1	3.96	58.7	44.61 ± 0.39^{d}	17.92 ± 0.34^{c}	0.00	21.67 ± 0.47^{ab}
13	180	40	25:1	3.96	59.0	44.35 ± 0.25^{d}	18.46 ± 0.62^{c}	0.00	22.33 ± 0.47^{abc}
3	180	60	20:1	4.13	56.6	48.87 ± 1.01^{e}	15.41 ± 0.26^{b}	0.00	26.67 ± 0.94^{de}
8	180	60	30:1	4.13	58.7	46.03 ± 1.73^{de}	18.48 ± 0.36^{c}	0.28 ± 0.40	25.00 ± 0.47^{ab}
10	200	20	25:1	4.25	51.4	64.16 ± 3.24^{g}	0.00 ± 0.00^{a}	0.00	26.00 ± 0.00^{cde}
1	200	40	20:1	4.55	51.8	63.54 ± 4.20^{g}	0.00 ± 0.00^{a}	0.00	29.00 ± 0.47^{e}
2	200	40	30:1	4.55	52.3	65.19 ± 1.20^{g}	0.00 ± 0.00^{a}	0.00	24.67 ± 0.94^{bcd}
17	200	60	25:1	4.72	50.1	58.71 ± 1.24^{f}	0.00 ± 0.00^{a}	0.00	28.33 ± 0.47^{e}
NP	-	-	-	-	100	30.27 ± 0.15^{a}	20.81 ± 0.15^{d}	2.68 ± 0.05	24.65 ± 0.54^{bcd}

843 ^{abcdefg} means the statistical significance.
844

Table 3. Chemical composition of liquid fraction from liquid hot water pretreated Hybrid *Pennisetum*.

Components of hydrolysate	Pretreatment experiment No.																
	5	16	15	11	14	7	4	6	9	12	13	3	8	10	1	2	17
Degradation products (g/L)																	
Acetic acid	0.30	0.32	0.23	0.29	0.48	0.34	0.51	0.54	0.52	0.50	0.53	0.85	0.48	0.67	1.33	0.79	0.87
Glucuronic acid	0.28	0.28	0.21	0.23	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.27	0.15	0.30	0.39	0.49	0.23
Glycolic acid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	1.07	0.42	0.45
Formic acid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.73	N.D	0.21
Levulinic acid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.16	N.D	0.16
Sugars (g/L)																	
Total glucose	2.13	1.95	1.32	1.82	1.99	1.45	1.68	1.64	1.66	1.66	1.65	1.94	1.35	1.46	1.12	0.91	0.84
Glucose oligomer	1.20	1.02	0.68	0.95	1.25	0.78	1.10	1.05	1.07	1.08	1.07	1.46	0.86	1.01	0.62	0.59	0.38
Glucose monomer	0.93	0.93	0.64	0.87	0.75	0.67	0.58	0.59	0.59	0.58	0.58	0.48	0.49	0.44	0.50	0.32	0.46
Arabiose	0.42	0.66	0.35	0.78	1.27	0.58	1.24	1.23	1.21	1.23	1.22	1.54	0.85	1.00	N.D	N.D	N.D

N.D: Not detected.

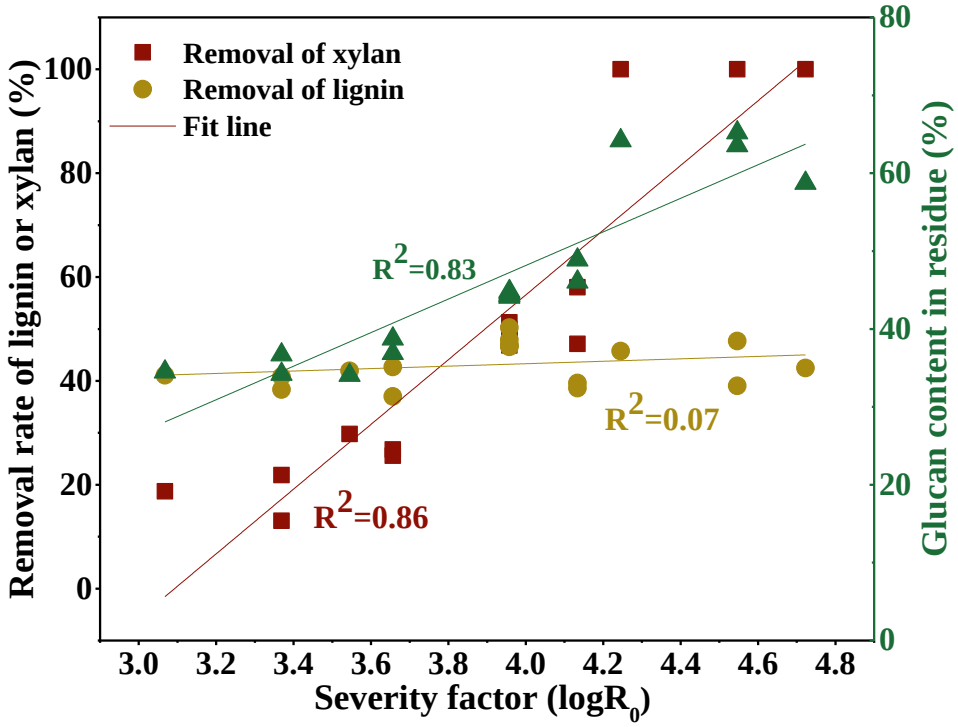
Table 4. The variance for the regression quadratic model equation of central composite design.

Source	Sum of squares	Degree of freedom	Mean square	F	Prop(P)>F
Model	11729.59	9	1303.29	4.49	0.0301
A-Temperature	3659.40	1	3659.40	12.62	0.0093
B-Reaction time	701.25	1	701.25	2.42	0.1639
C-Liquid-to-solid ratio	126.41	1	126.41	0.44	0.5302
AB	1521.00	1	1521.00	5.24	0.0558
AC	23.52	1	23.52	0.081	0.7840
BC	107.12	1	107.12	0.37	0.5625
A^2	4465.18	1	4465.18	15.40	0.0057
B^2	510.86	1	510.86	1.76	0.2261
C^2	255.51	1	255.51	0.88	0.3792
Residual	2030.02	7	290.00		
Lack of Fit	1928.99	3	643.00	25.46	0.0046
Pure Error	101.03	4	25.26		
Cor total	13759.61	16			
R^2 = 0.8525 Adjusted R^2 = 0.6628					
CV = 6.43% Adequate precision = 7.184					
Predicted residual sum of squares 31021.74					

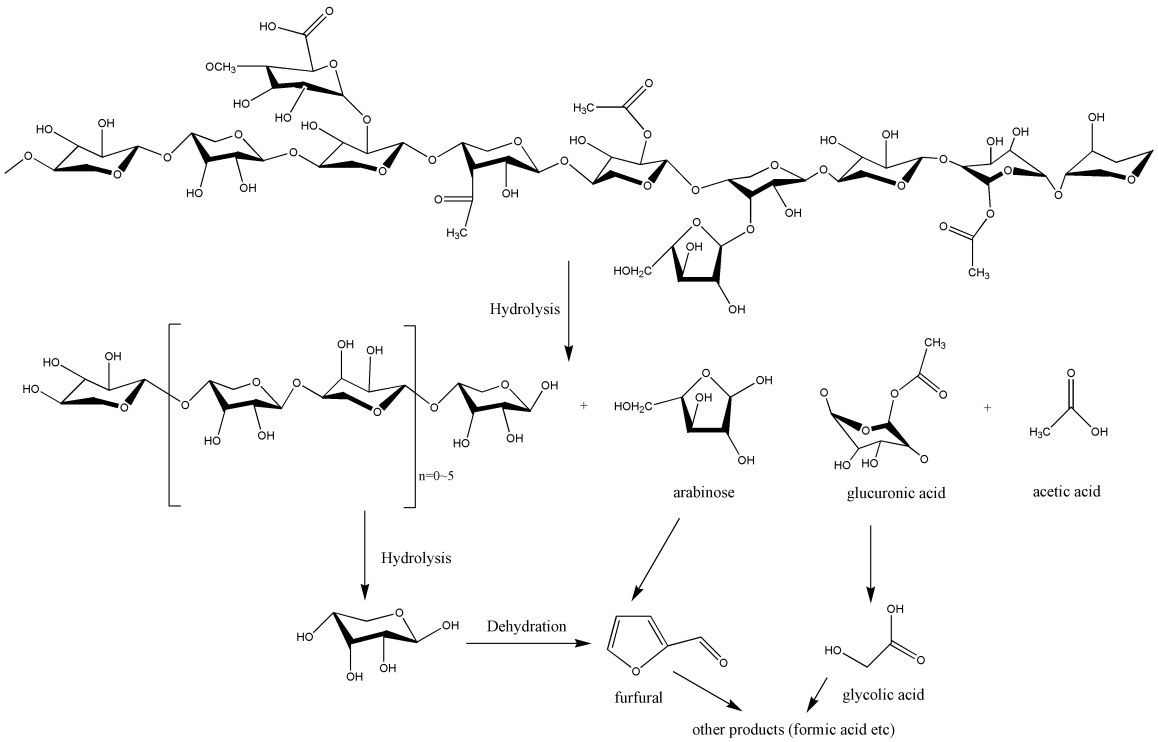
Table 5. The process dynamics of untreated and optimized liquid hot water pretreated Hybrid *Pennisetum*.

Substrate	Untreated HP	175℃ 35min treated HP	Cellulose
BMP yield (mL CH_4/g VS)	218.6[a]	290.6[b]	324.85
Theoretical yield (mL CH_4/g VS) [A]	349.1	349.1	413.9
BI (%) [B]	62.6	83.2	78.5%
Y_m (mL/g VS)	217.9	291.9	326.2
k ($days^{-1}$)	0.23	0.24	0.26
T_{50} [C]	3.01	2.84	2.68
R^2	0.994	0.992	0.969

[A] Theoretical methane yield is adopted from previous work;
[B] Biodegradability index = BMP yield/Theoretical yield × 100;
[C] T_{50} means the time needed to achieve the half of BMP yield;
[ab] means the statistical significance.

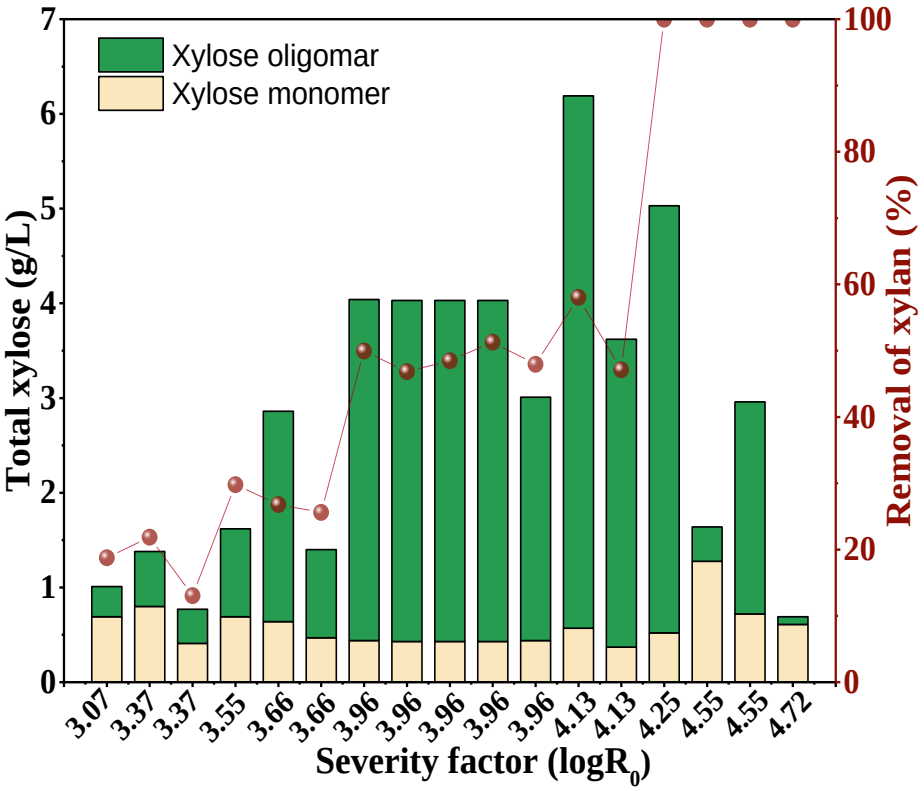


858
859 Figure. 1. The correlation between severity factor and chemical composition of
860 Hybrid *Pennisetum*.
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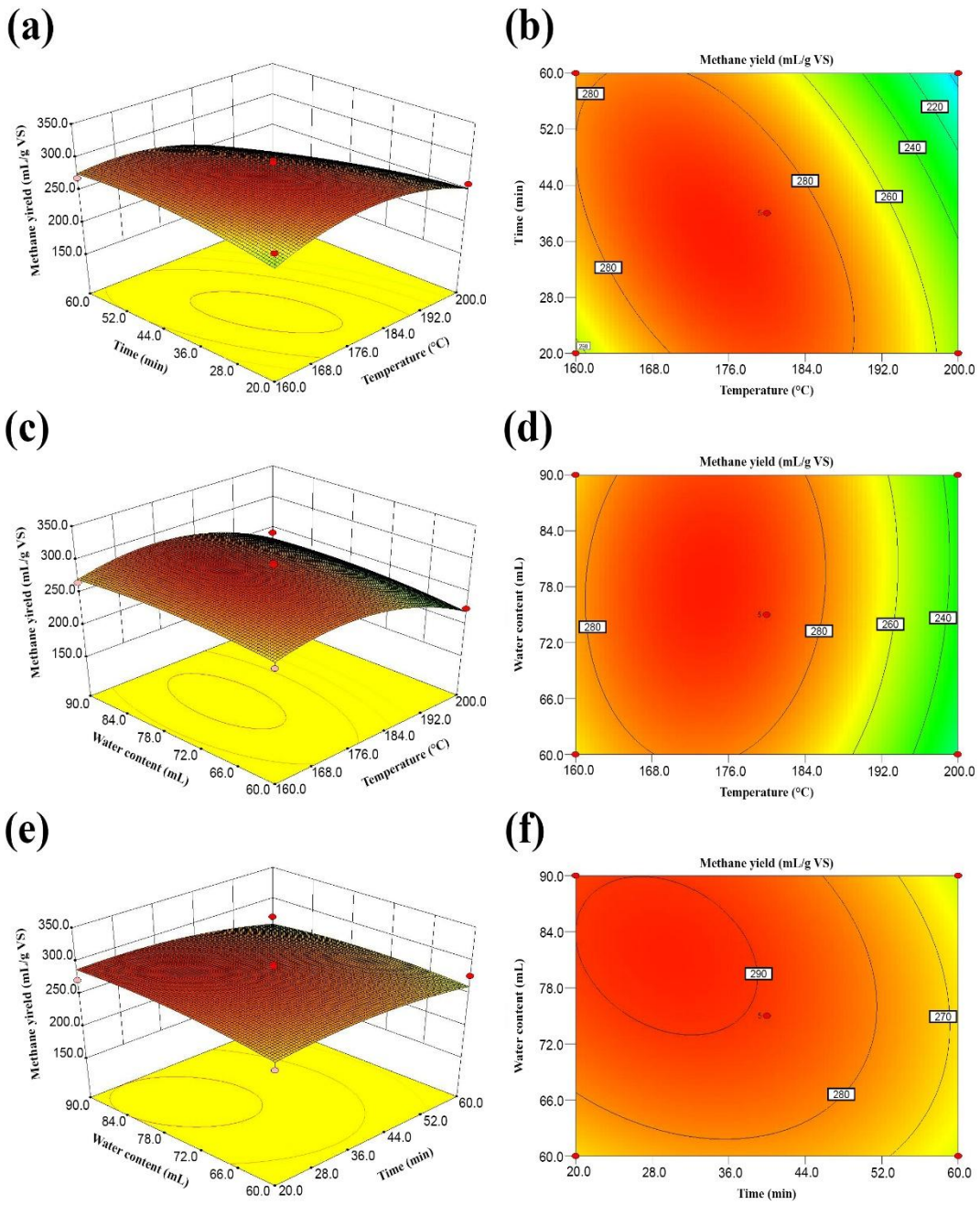
862
863 Figure. 2. Proposed pathway for *O*-acetyl-4-*O*-methyl-glucuronoarabinoxylan
864 decomposition in liquid hot water pretreatment modified from Yu et al. [58].
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867

868 Figure. 3. Concentration of xylose monomer and xylose oligomer in the liquid
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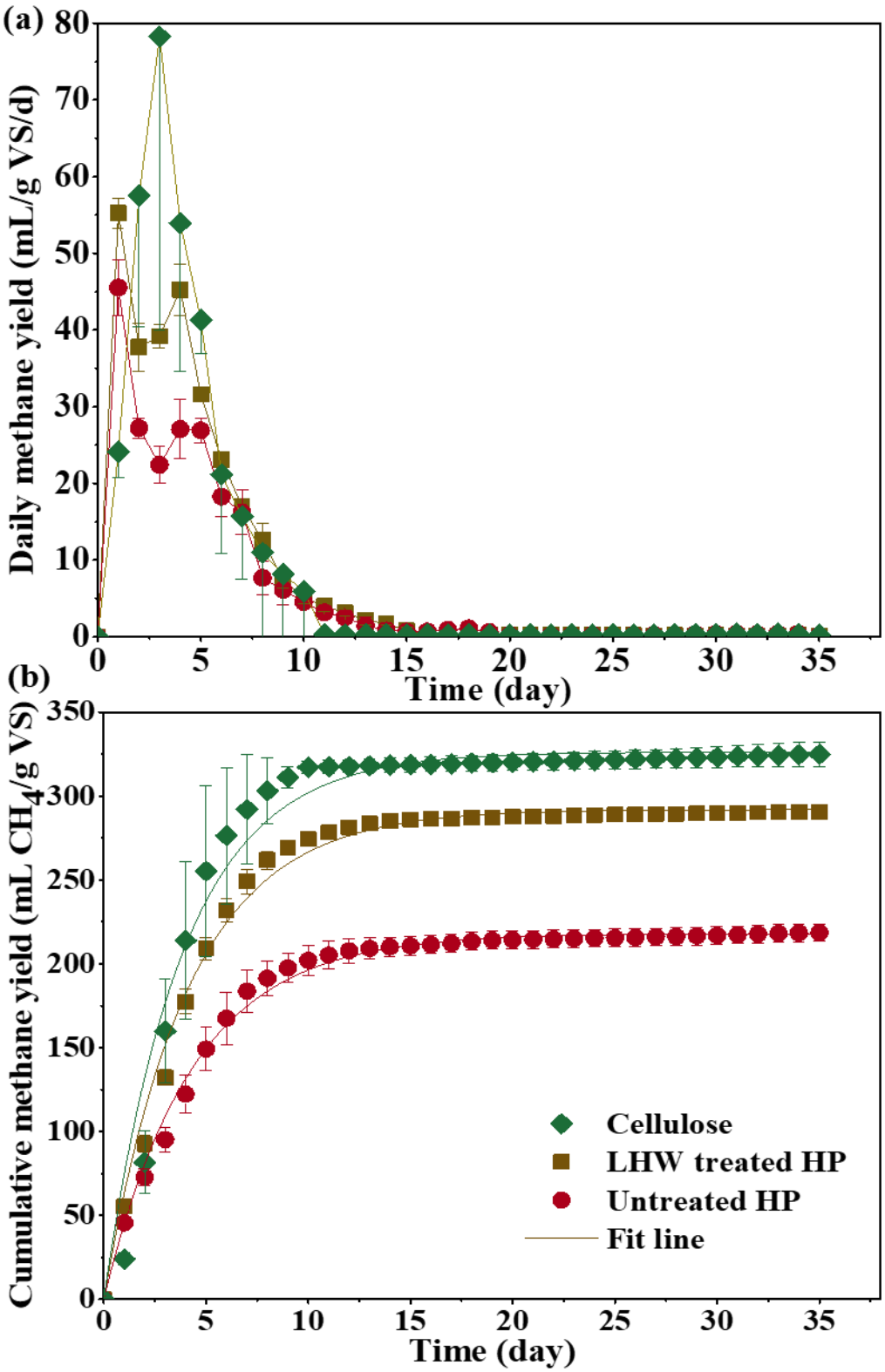


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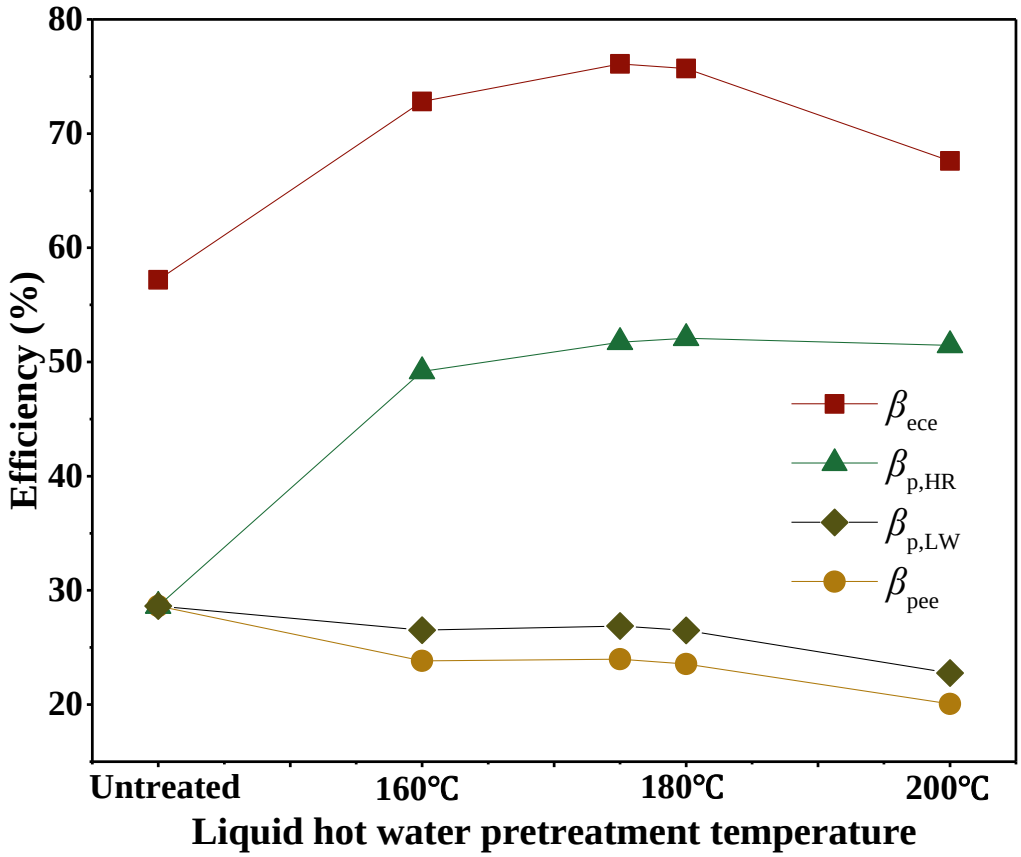
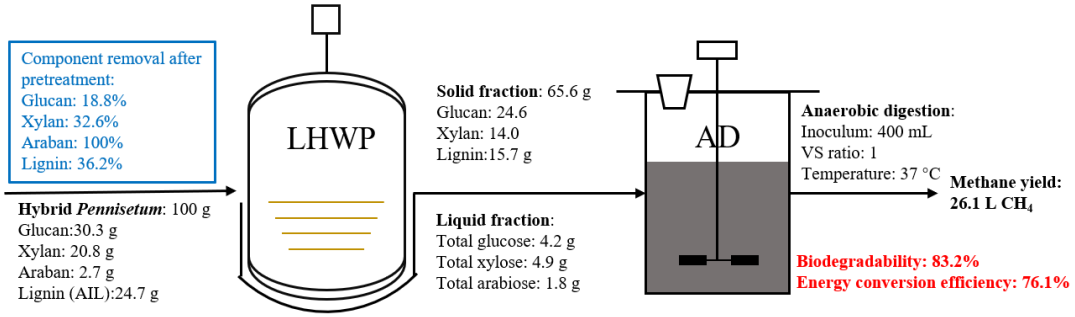


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