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1 Optimization of liquid hot water pretreatment on Hybrid Pennisetum

2 anaerobic digestion and its effect on energy efficiency

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18	Abstract: In this study, a central composite surface response methodology was used
19	to optimize the process variables (temperature, reaction time and liquid-to-solid ratio)
20	in liquid hot water pretreatment for biomethane production from the anaerobic
21	digestion of Hybrid Pennisetum (a typical energy crop in southern China).
22	Temperature, reaction time and water content were varied between $160 - 200$ °C, $20 - $
23	60 min and $60 - 90 mL$ respectively. The biomethane yields from pretreated material
24	were considered as response variables to different processes conditions. Results
25	showed untreated Hybrid Pennisetum had a biomethane potential of 218.6 L/kg
26	volatile solid (VS) (corresponding to a biodegradability of 62.6%). The effect of
27	liquid hot water pretreatment on the biomethane yield was diverse. Pretreatment
28	severities below 4.55 resulted in increased biomethane yields. However, further
29	increasing the severity resulted in lower biomethane yields. The optimum biomethane
30	yield (290.6 L/kg VS) was obtained at 175 °C and 35 min, with the highest
31	biodegradability of 83.2%. Energy balance showed that, under the optimal
32	pretreatment condition, the highest theoretical energy conversion efficiency of 76.1%
33	was achieved. If the process energy inputs are included, a process energy efficiency of
34	51.7% could be realized with proper heat recovery.
35	Keywords: Energy crops; Pretreatment; Liquid hot water; Anaerobic digestion;
36	Response surface methodology; Energy efficiency.
37	

38 **1. Introduction**

39 The transition to a sustainable and bio-based economy is crucial to reduce the 40 dependence on fossil fuels-based energy as it is non-renewable and produces 41 greenhouse gases such as CO₂ [1]. Bioenergy, produced from lignocellulosic biomass, 42 is believed to play an important role in the transition progress and has attracted 43 widespread attention [2], [3], [4]. 44 45 Lignocellulosic biomass, mostly composed of cellulose, hemicellulose and lignin, 46 including agricultural residues (such as rice straw and wheat straw), forestry residues 47 and energy crops [5], [6], [7], [8], is a promising resource for biofuel production 48 because it does not produce extra CO_2 as the CO_2 , released in subsequent uses, was 49 absorbed by the plant during growth [9]. Energy crops, such as switchgrass, elephant 50 grass and *Miscanthus*, have been intensively investigated for bioenergy production 51 due to its high dry matter yield, stress resistance (such as drought tolerance, cold 52 resistance and heavy metals endurance) and low nutrients requirement [10]. However, 53 a low biofuel production after biological conversion is always obtained due to the 54 inherent recalcitrance of lignocellulosic biomass; for example, the biomethane yields previously achieved were between 20% and 60% of the theoretical yield from various 55 substrates [9], [11], [12], [13]. Therefore, pretreatment prior to the biological 56 57 conversion process is recognized as an efficient way to improve biofuel production. 58

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

80	different biomass, there is no uniform LHW pretreatment conditions for different
81	types of lignocellulosic biomass [22], which necessitates the optimization of LHW
82	pretreatment conditions for a specific substrate.
83	
84	Hybrid <i>Pennisetum (Pennisetum americanum×P. purpureum</i> , HP), a C ₄
85	photosynthesis perennial grass, has recently received considerable interest for
86	bioenergy production because it can grow in marginal lands and has a high dry
87	biomass yield with a high cellulose and hemicellulose content of up to 60% [23]. It is
88	widely planted in south China, northeast China and the middle-lower Yangtze Plain
89	[24], and its annual dry matter yield is estimated at $60 - 130$ tons/hm ² [25]. It has been
90	proved that HP is a promising substrate for bioethanol and biomethane production
91	[26], [27]. Without pretreatment, the biomethane production from the AD of HP was
92	$208 - 249 \text{ L CH}_4/\text{kg VS}$, which increased to $288 - 302 \text{ L CH}_4/\text{kg VS}$ after physical or
93	chemical pretreatment, equivalent to an increase of 8 – 39% [23], [28], [29].
94	
95	It has been well known that lignin is a barrier in the AD process for methane
96	production from lignocellulosic biomass, and its removal can greatly improve the
97	methane production [29]; meanwhile, the removal or dissolution of hemicellulose is
98	also believed to have a positive impact on the methane production as the covalent
99	interactions between lignin and hemicellulose are a hurdle for downstream utilization
100	[30]. Yu et al. [31] reported that LHW pretreatment effectively facilitated the

101	enzymatic digestibility of HP by 67% after up to 98% of hemicellulose was removed.
102	However, the study associated with the effects of LHW pretreatment on the AD of HP
103	is not investigated. In an effort to understand the correlation between enzymatic
104	digestibility and anaerobic biodegradability (ABD) of lignocellulosic biomass, Jung et
105	al. [32] reported that there was an unreliable linear relationship ($R^2 = 0.70$) between
106	enzymatic digestibility and ABD, which was also observed by Frydendal et al. [33].
107	Moreover, Wang et al. [34] found higher pretreatment temperature (200 °C), even with
108	almost complete removal of hemicellulose, issued in decreased methane production
109	from rice straw. Therefore, in order to exploit the optimum biomethane production
110	from HP, it is essential to investigate the optimal LHW pretreatment conditions for the
111	AD of HP; as the LHW pretreatment is an energy intensive method, the energy
112	conversion efficiency and process energy efficiency of the integrated system of LHW
113	pretreatment and anaerobic digestion need to be evaluated for its practical application.
114	Herein, the objective of this study is to (1) evaluate the effect of LHW pretreatment on
115	the chemical composition and structure of HP, (2) optimize the LHW pretreatment
116	conditions to achieve the optimal methane production using response surface
117	methodology and (3) assess the energy efficiency of the integrated LHW pretreatment
118	and AD system by computing energy balance and energy process efficiency.
119	
120	2. Materials and methods
121	2.1 Material and inoculum

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

123	2016. The raw and fresh material was first cut into 2-3 cm, and then smashed for 1
124	min in a pulverizer (XB-HP, Xiaobao, China). The smashed samples were dried at 85 $^{\circ}$ C
125	for 3 days, and the dried samples were sieved with sieve mesh. The granulometric
126	fraction (20~80 mesh) was stored in a desiccator at room temperature before use.
127	
128	The characteristics of raw material and inoculum are shown in Table 1. Inoculum was
129	derived from a mesophilic continuously stirred tank reactor fed with cellulose and
130	grass. Before use, the inoculum was degassed for 7 days.
131	
132	2.2 Liquid hot water pretreatment
133	Liquid hot water (LHW) pretreatment was conducted in a 100 mL Teflon-lined
134	stainless autoclave reactor (Dalian Tongda Science and Technology Development Co.,
135	Ltd. China), which can stand a temperature of up to 300 °C and a pressure of 22 MPa.
136	During the LHW pretreatment, process parameters including temperature, time and
137	liquid-to-solid (L/S) ratio were investigated. The three parameters used in this study
138	were based on the research from Yu et al. [31] who reported that the optimal
139	pretreatment condition was 180 °C 40 min for HP, in which the enzymatic
140	digestibility was improved by 66.9% and pentose recovery was improved to 98.1%,
141	respectively. Therefore, the pretreatment temperature and reaction time used as the
142	central points for surface response design in this study was chose to be 180 °C and 40
143	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 \pm 0.5 °C
164	in an electric-heated thermostatic water bath and lasted for 35 days.

166 **2.4 Analytical methods**

167 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 168 169 (SEM) image was described before [28]. The chemical composition of untreated and 170 LHW pretreated samples was analyzed using two-step sulfuric acid hydrolysis method 171 according to Sluiter et al. [37]. The liquid fraction, collected from the washing process 172 after pretreatment, was used to measure the quantification of sugars (including 173 monomeric sugars, oligomeric sugars and total sugars), organic acids and 174 hydroxymethyl furfural (HMF) in the pretreatment hydrolysates. The measurement 175 was conducted by HPLC (Waters e2695) using a Shodex sugar SH-1011 column 176 coupled with refractive index detector and UV detector. The column temperature was 177 50 °C with a mobile phase of 0.005M H₂SO₄ at a flow rate of 0.5 ml per minute. The 178 quantification of total sugars was determined after a secondary hydrolysis with 4% 179 (w/w) sulfuric acid at 121 °C for 60 min as previously reported [31]. 180 2.5 Experimental design and analysis 181 182 As aforementioned, the pretreatment experiment was designed using a central composite response surface methodology (RSM) with 3 central point using Design 183 184 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, 185

 $186 \quad 20-60 \text{ min and } 20:1-30:1 \text{ respectively. All other process parameters such as heating}$

187	rate, the rate of stirring and the mass concentration were kept constant for each
188	experiment. This resulted in 17 experimental runs as shown in Table 2. Methane yield
189	was used as response variables to evaluate the effect of the process parameters in the
190	LHW pretreatment process. The severity factor $(log R_0)$, a measure of the pretreatment
191	intensity in hydrothermal process, which is a function of temperature (T) and reaction
192	time (t) was calculated using Eq. (1) (Ferreira et al., 2013).
193	$\log R_0 = \log(t \times \exp((T - 100)/14.75)) $ (1)
194	
195	The data from the BMP assays of the pretreated samples were used to fit the quadratic
196	equations generated by Design Expert 11 (Eqs. (2)).
197	$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} A B + \zeta_{23} B C + \zeta_{13} A C $ (2)
198	where Y represents the response variable for methane yield (mL/g VS); ζ_0 , ζ_1 , ζ_2 , ζ_3
199	mean coefficients of linear expressions; ζ_{11} , ζ_{22} , ζ_{33} are the quadratic coefficients and
200	ζ_{12} , ζ_{23} , ζ_{13} are interaction coefficients; A, B and C are the independent variables,
201	namely, pretreatment temperature, reaction time and L/S ratio. Then, the predicted
202	optimal values were confirmed by the verification tests using the adjusted values of
203	the three variables.
204	
205	The process dynamics, including decay constant (days ⁻¹) and maximum yield (Y_{max})
206	on the basis of BMP results from untreated and optimized LHW pretreated substrates,
207	were assessed by a first order differential equation (Eq. (3)). T_{50} (half time) was
	10

208	defined as the time needed to achieve 50% of the maximum cumulative biomethane	e
209	production. The biodegradability index (BI) was the ratio of BMP yield to the	
210	theoretical methane yield.	
211	$Y(t) = Y_m \times (1 - e^{(-kt)})$	(3)
212	Where t is the digestion time (days); k means the decay constant, which presents the	e
213	rate of degradability of the substrate; $Y(t)$ is the cumulative methane yield (mL CH ₄	/g
214	VS) at time <i>t</i> (days); Y_m refers to the maximum methane yield (mL CH ₄ /g VS).	
215		
216	2.6 Energy balance	
217	2.6.1 Process energy input	
218	The process energy inputs comprise the energy use in LHW pretreatment and AD. 7	The
219	total energy needed (E_{in} ; kJ/g fresh weight) can be reckoned using Eq. (4) modified	
220	from Lin et al. [38]:	
221	$E_{in} = E_{LHW} + E_{AD}$	(4)
222	Where E_{LHW} is the energy input for LHW pretreatment and E_{AD} is the energy input f	for
223	AD. The energy input for each process can be estimated by Eq. (5):	
224	$E_{LHW}/E_{AD} = (V_{reac}\rho_w Cp_w + m_{s,fresh}Cp_{s,fresh}) \times (T_{reac} - T_{room})$	(5)
225	Where the liquid fraction in the reactor (V_{reac}) is presumed as water with a heat	
226	capacity (Cp_w) of 4.18 kJ/kg/K. The LHW pretreatment temperature (T_{reac}) is chose	n
227	to be 160, 175, 180 and 200 °C in which the V_{reac} was the same to guarantee the	
228	temperature was the only independent variable; the room temperature (T_{room}) is	
229	assumed as 25 °C. The temperature of AD is 37 °C according to the experimental 11	

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)	
$Cp_{biomass} = 5.340Tp - 299 \tag{6}$	5)
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 a	IS
per Eq. (7):	
per Eq. (7): $E_{CH_4} = V_{CH_4,VS} \times HV_{CH_4} $ (7)	7)
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	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) $Cp_{biomass} = 5.340Tp - 299$ (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 a

251
$$\beta_{\text{pee}} = (E_{CH4} + E_{recov})/(E_{LHW} + E_{AD} + E_{HP}) \times 100\%$$
 (10)

252	where E_{recov} is the probable heat recovery from LHW pretreatment (this would be 0 as
253	per the experimental set-up). The heat recovery was calculated considering cooling
254	down the pretreatment hydrolysates from process temperatures to 50 $^{\circ}$ C [38] with an
255	assumed efficiency of 90% of a heat exchanger [42]. No heat recovery is obtained as
256	per the batch experimental set-up. However, as the LHW pretreatment is operated at
257	elevated temperatures, cooling down of the pretreated HP hydrolysates would provide
258	a promising opportunity for heat recovery with its subsequent use in providing heat
259	for AD.
260	
261	2.7 Statistical analysis
262	The data of chemical compositions and specific methane yields of untreated and LHW
263	pretreated samples were statistically analyzed utilizing SPSS 22.0 software with
264	one-way analysis of variance (ANOVA) at a probability level of 0.05.
265	
266	3. Results and discussion
267	3.1 Effects of LHW pretreatment on the chemical compositions of Hybrid
268	Pennisetum
269	3.1.1 Effect of LHW pretreatment on chemical composition of solid fraction
270	Solid recovery is an important parameter of consideration regarding to AD; higher
271	temperatures in LHW pretreatment would result in an increase of lignin content in the
272	recovered solid fraction which may lead to a lower overall methane yield [43]. Table 2

273	displays the solid recovery and chemical composition of untreated and LHW
274	pretreated HP. Overall, the solid recovery decreased as the temperature and reaction
275	time increased mainly due to the removal of xylan; while the L/S ratio had
276	insignificant effects on the solid recovery, which agreed with Perez et al. [44] who
277	observed that the solid recovery of LHW pretreated wheat straw decreased with
278	elevated temperature and reaction time. For example, with the same pretreatment time
279	of 60 min, the solid recovery of HP reduced from 69.2% to 56.6% and further
280	decreased to 50.1% as the temperature increased from 160 °C to 180 °C and further
281	increased to 200 °C; meanwhile, with the same pretreatment temperature such as
282	180 °C, the solid recovery was reduced from 71.8% to 56.6% as the reaction time
283	increased from 20 min to 60 min. However, it was found that there were no significant
284	effects of L/S ratio on the solid recovery when the temperature and reaction time were
285	set as the same. Li et al. [45] reported that the solid recovery of HP was 65.9% after
286	LHW pretreatment at 180 °C for 60 min with a L/S ratio of 10:1, and attributed the
287	solid loss to the removal of hemicellulose as it accounted for 67.7% of the total mass
288	loss.
289	

290 LHW pretreatment is efficient in dissolving hemicellulose while producing less 291 inhibitory compounds compared to other chemical pretreatments [22]. As shown in 292 Fig. 1, the hydrolysis efficiency of xylan was relatively low in three low-intensity 293 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 °C when applying LHW pretreatment to energy
297	sorghums. As the severity increased to medium conditions (log $R_0 < 4.2$), 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 $^{\circ}$ C 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 °C, 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 °C 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 °C. 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			

336	productions during the pretreatment process [53]. The mechanism of hemicellulose			
337	removal during LHW pretreatment has been well elucidated in a previous review [54];			
338	the authors demonstrated that besides the hemicellulose removal, organic acids such			
339	as acetic acid and formic acid are generated. These organic acids, the desirable			
340	intermediates for AD, can be effortlessly converted into biomethane by methanogenic			
341	archaea [55]; it was also reported that these organic acids in turn facilitated the			
342	pretreatment [56].			
343				
344	3.1.2 Effect of LHW pretreatment on chemical composition of liquid fraction			
345	Above analysis illustrated that after LHW pretreatment, the main compositions of the			
346	solid residue were cellulose and lignin, while hemicellulose-derived degradation			
347	products existed in the liquid fraction. Table 3 presents the chemical components of			
348	the liquid fraction. Overall, the liquid fraction contained oligosaccahrides,			
349	monosaccharides (glucose, xylose and arabiose) and organic acids (acetic acid,			
350	glucuronic acid, glycolic acid, formic acid and levulinic acid), the majority of which			
351	can be derived from the hemicellulose composition in HP. Given the respective			
352	content of xylan and araban in the raw material is 20.8% and 2.7%, it can be			
353	concluded that the structure of the hemicellulose from HP is an arabinoxylan;			
354	moreover, considering the chemical composition in the liquid fraction, the			
355	arabinoxylan could be mainly in the form of			
356	O-acetyl-4-O-methyl-glucuronoarabinoxylan, which is the typical structure of			

357	hemicellulose in Gramineous biomass such as Vetiver grass [57] and sweet sorghum			
358	[58]. Fig. 2 exhibits the structure of O-acetyl-4-O-methyl-glucuronoarabinoxylan and			
359	its proposed decomposition pathway in LHW pretreatment; it consists of			
360	β -1,4-D-xylan backbone where <i>O</i> -2 is substituted by α -D-glucuronic acid residue, and			
361	<i>O</i> -3 is substituted by α -L-arabinose residue and acetyl group [57], [58]. The inhibitory			
362	compounds such as furfural and hydroxymethyylfurfural were not detected in all the			
363	experiments. The total glucose in the liquid fraction showed a tendency of decrease			
364	from 2.13 g/L to 0.84 g/L as the temperature increased from 160 to 200 °C, indicating			
365	the further degradation of glucose under severe conditions. However, the			
366	concentration of arabinose increased from 0.35 g/L to 1.54 g/L with the temperature			
367	increased from 160 to 180 °C, indicating that the hydrolysis effacy of xylan was			
368	strenghthened by increased temperature due to the formation of hydronium ions			
369	(H_3O^+) . It was well elucidated that hemicellulose removal efficiency increased with			
370	elevated temperature and longer retention time, which was mostly solubilized as			
371	oligomers during LHW pretreatment [51], [59]. The concentration of			
372	xylooliglosaccharides and xylose is presented in Fig.3. At 6 low-intensity tests ($logR_0$			
373	< 4.0), the concentration of total xylose (xylose oligomer and xylose) was low			
374	(0.75~1.61 g/L), confirming the limitation of LHW pretreatment on dissolving			
375	hemicellulose at low temperature and short reaction time. The concentration of total			
376	xylose at medium-intensity ($4.0 < \log R_0 < 4.5$) was higher than that in low-intensity			
377	tests, which was 4.02–6.30 g/L. While under the harsh conditions ($\log R_0 > 4.5$), the			

378	concentration of total xylose decreased to 0.65–2.95 g/L due to the further degradation			
379	of xylose to the organic acids such as formic acid, which can be confirmed by the			
380	subsequent analysis of organic acids. Phuttaro et al. [4] observed similar results when			
381	treating Napier grass by LHW pretreatment. Li et al. [45] disclosed that the degree of			
382	polymerization of xylooligosaccharides in LHW pretreatment hydrolysate was less			
383	than 6, pointing out that under the same LHW pretreatment condition, the hydrolysis			
384	efficiency of hemicellulose in HP was higher than that in switchgrass and Miscanthus.			
385				
386	At 160 °C, the main organic acids were acetic acid (0.23–0.32 g/L) and glucuronic			
387	acid (0.21–0.28 g/L), while acetic acid was the main organic acid at 180 °C (Table 2).			
388	Acetic acid was produced from the acetyl group in			
389	O-acetyl-4-O-methyl-glucuronoarabinoxylan in LHW pretreatment [46] and it can be			
390	concluded from a forementioned results that under 180 °C, the hemicellulose in HP was			
391	hydrolyzed to oligoxylose, arabinose, glucuronic acid and acetic acid following the			
392	initial hydrolysis pathway in Fig. 2. As the temperature increased to 200 °C, the main			
393	organic acids were acetic acid (0.67–1.33 g/L) and glycolic acid (0.42–1.07 g/L).			
394	Glycolic acid was derived from glucuronic acid through the subsequent hydrolysis			
395	pathway as shown in Fig. 2 (the degradation mechanism of glucuronic acid can be			
396	found in supplementary material). It can be concluded that as the temperature			
397	increased, the autoionization process was enhanced and thereby intensified the			
398	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	

419 **3.2.1 Response surface model analysis**

Table 4 shows the analysis of variance for the regression quadratic model equation of 420 central composite design. The Model F-value of 4.49 implied the model was 421 422 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 423 significant. In this case, A and A^2 were significant model terms. The "Lack of Fit 424 425 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 426 determination coefficient (R^2) was used to measure the quality of the model equation 427 fitting and to decide the amount of variation explained by the model. In the observed 428 response values, the correlation coefficient, $R^2 = 0.8525$, demonstrated that the sample 429 430 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 431 432 precision measured the signal to noise ratio and a ratio greater than 4 was preferable. 433 The ratio of 7.184 indicated an adequate signal, meaning that this model can be used 434 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 435 436 higher the CV value, the lower the reliability of experiment. Hence, a lower value of 437 CV (6.43%) exhibited a greater reliability of the experiment. The predicted residual 438 sum of squares (PRESS) is a measure of how well the model fitted each point in the 439 design. The smaller the PRESS statistics, the better the fitness between the model and

440	the data points. Here the value of PRESS was found to be 31021.74 meaning the			
441	model well fitted the data points. Multinomial regression analysis was carried out on			
442	the experimental data, and the quadratic regression equation was shown as following:			
443	Y =			
444	288.98-21.39A-9.36B+3.98C-19.50AB+2.42AC-5.17BC-32.56A ² -11.01B ² -7.79C ²			
445				
446	The response surface of methane yield and the corresponding contour plots were			
447	displayed as Fig. 4. When the ratio of L/S was 25:1 (75 mL water: 3 g VS sample), the			
448	combined effect of temperature and time on methane yields was shown in Fig. 4(a-b			
449	From Fig. 4(a), the methane yields gradually increased with the increase of			
450	temperature and time while decreased as the temperature increased to 200 °C. The 3-J			
451	surface was steep, and the contour line tended to an ellipse, which all indicated that			
452	there was a significant effect of temperature on the methane production. From Fig.			
453	4(b), when the temperature and time were controlled at the range of $168 - 190$ °C and			
454	35-50 min, respectively, the methane yield would be higher than 280 mL/g VS. The			
455	maximum methane yield could be acquired within these range of pretreatment			
456	temperature and time.			
457				
458	When the pretreatment time was set at 40 min, the combined effect of temperature and			
459	L/S ratio on the methane yields was displayed at Fig. 4(c-d). From Fig. 4(c), the			
460	methane yield decreased at the temperature of $180 - 200$ °C while no significant effect			

461	of L/S ratio on the methane yields was observed. In addition, the response surface was			
462	relatively steep, and the contour line presented an ellipsis shape, which both			
463	demonstrated that the interaction between the two factors was remarkable. As			
464	depicted in Fig. 4(d), the methane yield was not lower than 280 mL/g VS between the			
465	temperature of $165 - 180$ °C and water of $70 - 85$ mL.			
466				
467	When the temperature was 180 °C, the combined effect of pretreatment time and L/S			
468	ratio on the methane yields was displayed at Fig. 4(e-f). From Fig. 4(e), the methane			
469	yield decreased at the pretreatment time of $35 - 60$ min. However, though the contour			
470	line presented an ellipsis shape, the response surface was relatively flat, which			
471	demonstrated that the interaction between the two factors was not notable. As depicted			
472	in Fig. 4(f), the methane yield was not lower than 290 mL/g VS between the			
473	pretreatment time of 20-45 min and L/S of 23:1-30:1.			
474				
475	The three kinds of process parameters as the key factors and the highest methane yield			
476	as the optimization objective was optimized by the RSM respond surface. According			
477	to the investigation, the predicted highest of methane yield could be 293.4 mL/g VS at			
478	the optimal condition of temperature of 175.5 °C, reaction time of 34 min, and L/S			
479	ratio of 26.6:1 (corresponding to the water content of 79.8 mL).			
480				

3.2.2 Confirmatory experiments and its kinetic study

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

502	In addition, it was found that the AD efficiency of Miscanthus was improved after
503	LHW pretreatment as the retention time of AD was decreased by 50% [10].
504	Kamusoko et al. [66] compared the efficacy of physical, chemical, biological and
505	combined pretreatment methods in enhancing biomethane production from crop
506	residues, and summarized that physical pretreatment method such as LHW
507	pretreatment was the most effective and fastest in terms of the rate of hydrolysis
508	during pretreatment. This further led to a shorter retention time of AD than that
509	without pretreatment. Therefore, it was necessary to understand the effect of LHW
510	pretreatment with optimal condition on the AD performance of HP.
511	
512	Fig. 5 and Table 4 display the parameters such as the kinetic decay, T_{50} and maximum
513	yield for understanding the process dynamics. Cellulose was used as a control to test
514	the activity of the inocula; the average methane production from cellulose was 324.9
515	mL/g VS-added, indicating that the inocula were in a good condition [67]. The first
516	order equation showed very high correlation as values of R^2 were 0.992 and 0.994 for
517	LHW pretreated and untreated HP, respectively. Both untreated and LHW pretreated
518	HP showed a good kinetic decay (k value was 0.23 and 0.24, respectively). The
519	highest daily methane yield was increased from 45.55 mL/g VS/d to 55.23 mL/g VS/d
520	at day 1 after LHW pretreatment with an increase of 21% (Fig.5 (a)). However, the
521	time for achieving the 50% of cumulative methane yield was not profoundly
522	shortened (from 3.01 days to 2.84 days after LHW pretreatment) (Table 4). This can

be attributed to the relatively good degradability of the untreated sample as its biodegradability was 62.6% which was higher than that from previous studies [29], [68]. This can be further explained by the results of inoculum accumulation as the inoculum used in this study was fed with grass for a long time. Gu et al. [69] reported that the inoculum has a great impact on the AD of biomass, and the authors suggested it is essential to conduct the adaptation step before AD assay to achieve the maximum 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

634 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,		

bowever, 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.

568

569 **4. Conclusion**

570 Liquid hot water pretreatment can not only alter the structure of Hybrid Pennisetum, 571 but also improve its anaerobic digestion performance under certain condition. LHW 572 pretreatment was proven to be efficient in dissolving hemicellulose, which resulted in 573 up to 100% of xylan removal of Hybrid Pennisetum. The optimal LHW pretreatment 574 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 575 576 Pennisetum led to a relatively short retention time in AD to achieve the 50% of total 577 methane production. Energy balance showed that the highest energy conversion 578 efficiency of 76.1% was achieved at the optimal pretreatment condition. The 579 maximum process energy efficiency of 28.6% was obtained from the untreated 580 substrate considering the energy input for pretreatment and anaerobic digestion. However, it can be significantly increased to 51.7% for the optimal pretreatment 581 582 condition with heat recovery. This provides fundamental information for further 583 system optimization and enables a possibility for energy crops utilization in an 584 integrated thermochemical and biological process.

585

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- 599

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810 **Table and figure captions:**

- 811 Table 1. Compositional characteristics of Hybrid Pennisetum and inoculum.
- Table 2. Chemical composition of untreated and liquid hot water pretreated hybrid
- 813 Pennisetum.
- Table 3. Chemical composition of liquid fraction from liquid hot water pretreated
- 815 hybrid *Pennisetum*.
- 816 Table 4. The variance for the regression quadratic model equation of central
- 817 composite design.
- Table 5. The process dynamics of untreated and optimized liquid hot water pretreated
- 819 hybrid Pennisetum.
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- Figure. 1. The correlation between severity factor and chemical composition of hybrid*Pennisetum*.
- Figure.2 Proposed pathway for O-acetyl-4-O-methyl-glucuronoarabinoxylan
- 824 decomposition in liquid hot water pretreatment.
- Figure. 3. Concentration of xylose monomer and xylose oligomar in the liquid
- 826 fraction after liquid hot water pretreatment.
- Figure. 4. Response surface analysis of methane yield from the anaerobic digestion of
- 828 liquid hot water pretreated Hybrid *Pennisetum* (R1: methane production; A, B and C:
- 829 temperature, reaction time and water use).
- Figure. 5. Daily (a) and cumulative (b) methane yield of untreated and optimal liquid
- 831 hot water (LHW) treated hybrid *Pennisetum*. Cellulose was used as control group to
- test the activity of the inocula.
- 833 Figure. 6. Energy conversion efficiency (β_{ece}) and process energy efficiency (β_{pee}) of
- 834 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
- 838 hot water pretreatment condition and anaerobic digestion.
- 839

<u> </u>	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	/

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

Experiment No.	Temperature (°C)	Time (min)	Liquid-to-subs trate 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}	$24.80\pm2.26^{\rm f}$	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	$18.83\pm0.10^{\rm c}$	0.00	22.33 ± 0.47^{abc}
9	180	40	25:1	3.96	58.4	44.08 ± 0.10^d	$18.18\pm0.06^{\rm c}$	0.00	22.33 ± 0.47^{abc}
12	180	40	25:1	3.96	58.7	44.61 ± 0.39^d	$17.92 \pm 0.34^{\rm c}$	0.00	21.67 ± 0.47^{ab}
13	180	40	25:1	3.96	59.0	44.35 ± 0.25^{d}	$18.46 \pm 0.62^{\rm c}$	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	$65.19 \pm 1.20^{\text{g}}$	$0.00\pm0.00^{\mathrm{a}}$	0.00	24.67 ± 0.94^{bcd}
17	200	60	25:1	4.72	50.1	$58.71 \pm 1.24^{\rm f}$	0.00 ± 0.00^{a}	0.00	28.33 ± 0.47^e
NP	-	-	-	-	100	30.27 ± 0.15^a	$20.81 \pm 0.15^{\text{d}}$	2.68 ± 0.05	24.65 ± 0.54^{bcd}

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

843 ^{abcdefg} means the statistical significance.

Components of							Pret	treatme	nt expe	eriment	No.						
hydrolysate	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

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

846 N.D: Not detected.

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

Source	Sum of	Degree of	Mean	F	Prop(P)>F
	squares	freedom	square		
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			
$R^2 = 0.8525$ Adjuste	ed $R^2 = 0.6628$	3			
CV = 6.43% Adequ Predicted residual s	ate precision = um of squares	= 7.184 31021.74			

851	Table 5. The process	dynamics of untr	reated and optimized	liquid hot water pretreated
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Hybrid Pennisetum.

Substrate	Untreated HP	175°C 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
$k (\mathrm{days}^{-1})$	0.23	0.24	0.26
T_{50}^{C}	3.01	2.84	2.68
\mathbf{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
859 Figure. 1. The correlation between severity factor and chemical composition of
860 Hybrid *Pennisetum*.



862 other products (formic acid etc)
863 Figure. 2. Proposed pathway for *O*-acetyl-4-*O*-methyl-glucuronoarabinoxylan

decomposition in liquid hot water pretreatment modified from Yu et al. [58].



868 Figure. 3. Concentration of xylose monomer and xylose oligomer 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*.



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



877 878 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 879

cooling down of pretreated hydrolysates. $\beta_{p,LW}$: Considering 30% less water use than 880 that in liquid hot water pretreatment without heat recovery. 881



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