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1	Improving methane production from <i>Pennisetum</i> hybrid by
2	monitoring plant height and ensiling pretreatment
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17	Abstract: The biomass of grass-based Pennisetum hybrid commonly use for
18	abiogas production via anaerobic digestion. However, it is necessary to determine
19	a method to optimize the plant harvest time for high biogas production. Moreover,
20	ensiling of biomass in the presence of diverse microbes may offer a solution to
21	improve biogas production. In this study, whole plant of Pennisetum biomass
22	(including stems and leaves) was collected at different harvesting time (plant
23	heights of 70, 100, 150 cm), and then comparatively assessed for further ensiling
24	and biogas production. Compared to leaves, stems exhibited a significant linear
25	relationship ($R^2 = 0.99$) with whole plants in terms of ensiling quality (i.e. pH and
26	NH ₃ -N). Microbial analysis further revealed that <i>Lactobacillus</i> was the dominant
27	bacterial genus during ensiling of stems and whole plants, with the highest relative
28	abundance of 50.08% obtained at the height of 100 cm. Ensiling of biomass at a
29	height of 100 cm achieved the best digestion performance, with the methane yields
30	of 316 ± 20 mL/g VS for leaves, 361 ± 43 mL/g VS for stems, and 356 ± 28 mL/g
31	VS for whole plants. A harvesting time at the plant height of 100 cm was the
32	optimal from the silage quality and anaerobic digestion performance.
33	Keywords: Pennisetum hybrid biomass; plant height; ensiling; Lactobacillus bacteria;

- 34 anaerobic digestion, methane.
- 35 Graphical Abstract







Pennisetum (subfamily: Panicoideae, tribe: Paniceae) is a genus of C4 grasses that are widely grown in Europe and Asia. ¹ *Pennisetum* sp. is economically feasible and recommended as a promising feedstock for anaerobic digestion, due to its huge biomass yield and high organics content. ²⁻⁴ The annual *Pennisetum* biomass yield was reported as 88 MT/ha, 210 t/ha of which were produced in China. ⁵ The organic components of *Pennisetum* biomass are mainly composed of cellulose (40–60%) and hemicellulose (20–40%), which can be easily degraded and used in biological process.¹

45 However, the use of *Pennisetum* biomass may not be straightforward. Plant harvest time is important for anaerobic digestion performance, because the chemical 46 composition of grass varies with its growth stage.^{6,7} For example, the specific methane 47 yields of *Pennisetum* hybrid and switchgrass (*Panicum virgatum*) decreased from 280 48 to 119 mL/g VS, ⁸ and from 266-309 to 191-250 mL/g VS as crops matured.⁹ 49 Lehtomaki et al.¹⁰ observed that harvesting at a younger stage was optimal for Napier 50 51 grass (Pennisetum purpureum) because it could achieve a higher specific methane yield, whereas marrow kale (Brassica oleracea var. medullosa) and Jerusalem artichoke 52

(helianthus tuberosus) were optimal at a later harvest, which could obtain higher 53 biomass yields. Dragoni et al.¹¹ reported that harvesting in September might be the 54 55 most feasible option for *Phragmites australis*. Similarly, the optimal cutting time for Miscanthus was between September and October.¹² In addition, Surendra and Khanal 56 ¹³ obtained a maximum methane yield of $219 \pm 4.9 \text{ mL/g}$ VS for *P. purpureum* 57 harvested at 2 months old. Overall, the optimal harvest time varies by species, growth 58 conditions, maturity stage, and planting area. Therefore, establishing a simple method 59 to determine the harvest time is necessary to enhance methane production. 60

61 Furthermore, the rigid cell wall structures in biomass are strongly recalcitrant to microbial degradation. Therefore, it is critical to pretreat the Pennisetum hybrid to 62 improve the specific methane yield. Compared to various pretreatments of biomass, 63 64 ensiling is a commonly used technology that can destroy the structure of cellulose and hemicellulose, and preserve the nutrient component as effectively as possible.¹⁴⁻¹⁸ High 65 quality silage can recover 87–98% of methane yield on the basis of methane potential 66 of the biomass.¹⁹ Vervaeren et al.²⁰ observed the process of silage could effectively 67 improve anaerobic digestion performance with an increase 10.1-14.7% of biogas 68 production. 69

However, to the best of our knowledge, few researches were reported about combining the aspect of harvest time and ensiling pretreatment to enhance methane production from *Pennisetum* hybrid. Therefore, the present study aimed to (1) improve the silage quality and anaerobic digestion performance by comparing grass at different heights; (2) evaluate the leaf and stem parts in whole plant to study the primary influencing component of the silage process and conversion efficiency; and (3)
conclude the feasibility of determining the optimal harvest time by monitoring plant
height.

78 **2.** Methods

79

2.1 Grass materials and inoculum

The biomass, Pennisetum hybrid, was sown in Zengcheng district, Guangzhou, 80 China. The *Pennisetum* hybrid planting spacing is $60 \text{ cm} \times 12 \text{ cm}$, and the planting area 81 is 1000 m² (50 m \times 20 m). Samples were collected at January 14, 2016, the 82 83 corresponding grasses at heights of 70 cm, 100 cm, and 150 cm were selected for the study. 5-10 strains were randomly selected from the experimental base for each 84 castration, leaving 10 cm for growth. Before processing the grass, the quality of fresh 85 86 whole plant was weighted. For the comparison of the main factors for determining the silage quality and anaerobic digestion performance, some of the raw materials were 87 separated and classified into leaves and stems, whereas other materials were classified 88 89 as whole-plant samples.

The inoculum for the anaerobic digestion was obtained from continuously stirred tank reactors operated in the lab. The total solids (TS) and volatile solids (VS) contents of the inoculum were determined as 3.44% and 1.43%, respectively.

93

2.2 Experimental setup and procedure

The fresh materials were cut into small pieces of 2-3 cm, pulverized, and then stored at -20°C in a refrigerator until further use. The silage materials were prepared in a plastic silo bag. For the ensiling process, about 200 g of fresh sample was placed in a

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bag, vacuumed-sealed, and then ensilaged at ambient temperature for 30 d. After
ensiling processing, the silage samples were crushed and then stored at -20°C in a
refrigerator for spare. Each treatment was performed in triplicate.

The batch anaerobic digestion experiments were carried out using an automatic methane potential test system (AMPTS II, Bioprocess Control Sweden AB) at 35 ± 1 °C., the total and working volume of reactor was 500 mL and 400 mL, respectively. In this process, 400 mL of inoculum were used, and the ensiling material was added based on the VS of substrate/inoculum ratio of 1. The experiments were performed in triplicate and were run for 30 d.

106 **2.3 Analytical methods**

107 The TS, VS, pH, total ammonia nitrogen concentration (NH₃-N), carbon (C), and 108 nitrogen (N) analyses were performed according to previously published methods. ¹⁶ To 109 determine the microbial community composition in silages of different materials, the 110 collected samples were stored at -20°C until the analysis. Microbial characterizations 111 were based on the method of 16s rRNA high throughput sequencing. The microbial 112 DNA was extracted, amplified, and analyzed according to a previously described 113 method. ²¹

114 **2.4 Kinetic analysis**

115

5 The modified Gompertz equation (Eq. (1)) was used for the kinetic analysis ²²:

116
$$M = P \times \exp\left\{-\exp\left[\frac{R_m \times e}{p}(\lambda - t) + 1\right]\right\}$$
(1)

117 where *M*, *P*, R_m , and λ represent the cumulative methane yield (mL/g VS) at a given 118 time, methane production potential (mL/g VS), maximum methane production rate 6 / 25 119 (mL/g VS d), and lag phase (d), respectively.

120 **3. Results and discussion**

121 **3.1 Chemical composition of the materials**

Pennisetum hybrid as the feedstock for anaerobic digestion mainly includes the 122 parts of stem and leaf, Table 1 presents the TS, VS, C, N, and C/N contents of the stem 123 and leaf in the whole plant obtained at different conditions. Fresh and silage samples 124 typically exhibited significant differences in terms of TS, VS, and N contents. Moreover, 125 samples of different plant parts derived from various plant heights (i.e. 70, 100, and 150 126 127 cm) also contributed to different chemical compositions. For the fresh materials, the TS contents increased from $13.91 \pm 1.09\%$ to $23.11 \pm 1.65\%$ in the whole plant, from 18.13 128 $\pm 0.10\%$ to 25.73 $\pm 1.08\%$ in leaf, and from 11.97 $\pm 0.57\%$ to 23.07 $\pm 0.03\%$ in stem as 129 130 the plant height increased. The increase in the TS and VS contents of Pennisetum hybrid showed a positive correlation with plant height. These results could be due to the total 131 lignocellulose (including cellulose, hemicellulose, and lignin) content increased with 132 crop maturity.²¹ Moreover, leaf had the highest TS and VS contents, whereas stem had 133 the lowest TS and VS contents in different samples of plant height. No significant 134 difference was observed in the C contents among different biomass parts and heights; 135 however, the highest N content was obtained in leaf and the lowest N content in stem. 136 Correspondingly, the C/N values were higher in stem than those in leaf. Similar trends 137 were previously observed by Erickson et al.²⁴ and Han et al.,²⁵ who reported that the 138 N concentration in sorghum leaf was higher than that in the stem. For the silage 139 materials, the content of TS, VS, and N contents had a decrease compared to the fresh 140

141	samples, whereas the corresponding C/N values showed an increase. Moreover, the N
142	content in whole plant silage materials decreased from 0.98 \pm 0.02% to 0.64 \pm 0.01%
143	with the plant height from 70 to 150 cm. The reason was that the process of ensiling
144	could degrade carbohydrates and proteins into minor molecular such as volatile fatty
145	acids (mainly including lactic acid, acetic acid, and propionic acid) and amino acids. ¹⁶
146	In addition, the lowest TS and VS contents were observed in the plant height of 100 cm
147	with different plant parts. Similarly to the fresh materials, higher TS, VS, and N
148	contents were observed in the leaf silage samples, corresponding to lower C/N values.
149	
150	Table 1.
151	
152	Figure 1 presents the pH values and NH ₃ -N concentrations in the silage samples
153	of the stem, leaf, and whole plant. In agreement with the N contents of stem, lower
154	NH ₃ -N concentrations of were obtained for stem silage samples. Meanwhile, lower pH
155	values of 4.15-4.49 were observed in the stem silage samples. By contrast, the leaf
156	silage samples had higher pH values of 4.73-5.54, which increased with plant height
157	from 70 to 150 cm. In addition, the NH ₃ -N concentrations in whole plant silage
158	materials decreased from 44.50 \pm 0.64 mg/L to 14.00 \pm 0.98 mg/L with the plant height
159	from 70 to 150 cm. Nousiainen et al. ²⁶ reported that a negative association was
160	observed between the decreasing crude proteins contend and the certain stage of plant
161	maturity. And the decreasing NH ₃ -N concentrations in the increasing heights of whole
162	plant silage materials were similar to the results of ammonia nitrogen in the dairy cow

163	fed silages harvested at four stages of grass maturity. ²⁷ The pH values of the stem and
164	whole-plant silage samples were similar to the so-called critical pH values (range: 4.10-
165	4.45) for silage samples at the dry matter of $15-30\%$. ²⁸ In order to understand the role
166	of plant part in the silage samples, the correlations of pH values and NH ₃ -N
167	concentration between the silages of stem, leaf, and whole-plant was analyzed in the
168	Figure 2. In a comparison of the pH values among the silage samples, a positive linear
169	relationship between stem and whole plant was observed, following the equation: y=
170	7.8226-0.7983x ($R^2 = 0.9987$). However, a negative linear relationship between stem
171	and whole plant was obtained by comparing the NH ₃ -N concentrations of silage
172	samples, and the equation was $y = -3.5975 + 1.3736x$ ($R^2 = 0.9994$). Although the same
173	linear relationship between leaf and whole plant was observed by comparing the pH
174	value and NH ₃ -N concentration of silage samples, there were not significant linear
175	correlation of pH ($R^2 = 0.0805$) and NH ₃ -H ($R^2 = 0.3601$) between the leaf and whole
176	plant. In addition, the stem accounted for over 60% of the content of fresh whole plant.
177	Therefore, these results suggested that the part of stem had a greater effect than the leaf
178	on the silage quality of the whole plant.

Figure. 1

Figure. 2

3.2 Bacterial community structure

184 Figure 3 presents the bacterial communities in the raw material and silage samples.

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The dominant bacterial compositions at the levels of phyla and genera were similar among the fresh materials. The dominant bacteria were *Cyanobacteria/Chloroplast*, with a relative abundance of 71.03–94.86%, and the major genus was *Streptophyta*, with a relative abundance of 71.03–97.96%.

In the silage samples, a dramatic shift in the bacterial compositions at the phylum 189 level was observed in comparison with those in the fresh materials. The relative 190 abundance of Cyanobacteria/Chloroplast decreased to 0.72-28.27%, whereas 191 Firmicutes (36.26-80.72%) and Proteobacteria (6.05-40.79%) became the dominant 192 193 bacteria at the phylum level after ensiling. Remarkable differences in the relative abundance at the phylum level were observed among the stem, leaf, and whole-plant 194 parts. Most sequences at the phylum level assigned to the genera Streptophyta, 195 196 Lactobacillus, Lactococcus, Raoultella, Enterobacter, Enterococcus, Leuconostoc, Serratia and Weissella. 197

The most dominant at the phylum level was Firmicutes, and a higher relative 198 199 abundance of *Firmicutes* was obtained in the stem and whole plant. Desirable functional bacteria in silage include Lactobacillus, Enterococcus, and Lactococcus, which are 200 used widely as silage additives. ²⁹ These bacteria belong to a major part of the lactic 201 acid bacteria group, which could convert sugars to lactic acid. ^{30, 31} Since lactic acid was 202 one of the main metabolic intermediates (VFAs) in process of anaerobic digestion, it 203 could easily utilize by the acetogenic bacteria and methanogens. ^{32,33} For the stem silage 204 samples, the relative abundance of Lactobacillus sp. ranged from 36.41% to 50.08%, 205 reaching a maximum at a height of 100 cm, while the relative abundance of Lactococcus 206

207	sp. decreased from 27.40% to 1.61% as height increased. This was coupled with an
208	increase in the relative abundance of the genus Enterococcus. In the leaf silage samples,
209	the relative abundance of Lactobacillus sp. ranged from 1.27% to 39.60%, reaching a
210	maximum at a height of 150 cm, while the variations in the relative abundance of the
211	genera of Lactococcus and Enterococcus were similar to those in the stem. In the whole
212	plant, the dominant genera differed by height. For example, Lactobacillus was the
213	primary genus at a height of 150 cm, while relative abundances of 37.62%
214	(Lactobacillus and Lactococcus) and 46.70% (Lactobacillus and Enterococcus) were
215	obtained for the silage samples at heights of 70 cm and 100 cm, respectively.
216	The other most abundant at the phylum level was Proteobacteria (6.05-40.79%),
217	the genera of Raoultella and Enterobacter predominated in this phylum. The relative
218	abundance of Raoultella in silage samples increased from 1.08% to 9.36% in stem
219	and from 0.71% to 30.73% in leaf, while the relative abundance in the whole plant
220	ranged from 2.42% to 24.52%. Enterobacter had a relative abundance of 0.55-
221	30.57%. Enterobacter and Raoultella have been shown to be deleterious
222	microorganisms during the ensiling process. ^{34, 35} Because these bacteria could largely
223	consume sugars and other simple compounds in ensiling process 34, 35 it is not
224	beneficial to produce more methane for anaerobic digestion. The lowest relative
225	abundance of Enterobacter and Raoultella in whole pant samples was obtained at a
226	height of 100 cm. Overall, the plant height of <i>Pennisetum</i> hybrid harvested at 100 cm
227	for ensiling not only had the highest relative abundance of desirable functional
228	bacteria (Lactococcus, Lactobacillus and Enterococcus), but also had the lowest

229	relative abundance of deleterious bacteria (Enterobacter and Raoultella) for ensiling.
230	Therefore, these results suggested that grass harvested at a plant height of 100 cm
231	could improve the quality of silage.
232	
233	Figure. 3
234	
235	3.3 Anaerobic digestion performance

Figure 4 and Table 2 present the cumulative and specific methane yields of fresh 236 237 and silage materials. For the fresh materials, the specific methane yields decreased from $238 \pm 12 \text{ mL/g VS}$ to $226 \pm 8 \text{ mL/g VS}$ for the whole plant and from $263 \pm 5 \text{ mL/g VS}$ 238 to 194 ± 10 mL/g VS for stem as height increased. Meanwhile, the 80% cumulative 239 240 methane yield was obtained at 9 d for the stem and whole plant at heights of 100 cm and 70 cm, respectively, but required 10-14 d for samples at a height of 150 cm. The 241 specific methane yields of leaf ranged from $206 \pm 5 \text{ mL/g VS}$ to $258 \pm 6 \text{ mL/g VS}$. 242 Ensiling decreased the time required to obtain an 80% cumulative methane yield to 7-243 12 d for different parts of grass. Moreover, an increased specific methane yield was 244 observed in the silage samples, and their specific methane yields were in the range of 245 263.17-298.04 mL/g VS, 315.75-361.25 mL/g VS, and 256.23-277.11 mL/g VS for the 246 plant height of 70 cm, 100 cm, and 150 cm, respectively. The maximum specific 247 methane yield of 316 ± 20 mL/g VS for leaf, 361 ± 43 mL/g VS for stem, 356 ± 28 248 mL/g VS for whole plant was obtained at a plant height of 100 cm. Since the 249 lignocellulosic structure of *Pennisetum* hybrid was disrupted by the desirable functional 250

bacteria in the process of ensiling, it could be efficiently converted into biogas by the 251 microorganisms of anaerobic digestion. ^{32, 33} In addition, the samples harvested at plant 252 253 height of 100 cm had a better silage quality by the bacterial community analysis. Similar specific methane yield results have been reported elsewhere. For example, the methane 254 yields for tall fescue, cocksfoot, and reed canary grass were between 238 mL/g VS and 255 446 mL/g VS depending on N fertilization and harvest frequency. ³⁶ Moreover, specific 256 methane yields of 135 mL/g VS and 185 mL/g VS were reported for switchgrass and 257 giant cane, respectively. ^{37, 38} The better performance of biogas production was observed 258 in the silage samples of the plant height 100 cm for preferred bacteria community. These 259 results suggested that harvesting plants at a height of 100 cm might be suitable for 260 biogas production from the perspectives of silage quality and anaerobic digestion 261 262 performance.

The regression analysis showed satisfactory overall agreements between the 263 experimental data and the model, with high regression coefficients ($R^2 > 0.94$) (Table 2 264 265 and Figure 4). More methane production potential and higher maximum methane production rate were observed in the silage samples. Similar result was observed in 266 anaerobic digestion of the silage Pennisetum purpereum with molasses-processed 267 wastewater addition.²¹ The stem, leaf, and whole plant from plants harvested at a height 268 of 100 cm were associated with a higher methane production potential and maximum 269 methane production rate compared with the silage samples harvested at heights of 70 270 cm and 150 cm. It indicated that the silage samples harvested at a height had a better 271 anaerobic digestion performance than the other ensiling samples. These predicated 272

273	results of the model were consistent with the specific anaerobic digestion performance
274	of the silage samples harvested at the height of 100 cm. A negligible lag time (λ) was
275	obtained for the fresh and silage samples. Allen et al. ³⁹ reported the biochemical
276	methane potential of hay grass varied from 156 mL/g VS to 433 mL/g VS for first cut
277	baled silage, and the kinetics analysis showed the similar results of the methane
278	production potential and lag time. According to the results of the specific methane
279	yields and the bacterial community analysis in the ensiling samples, the optimal
280	harvesting time at the plant height of 100 cm and the pretreatment of silage showed a
281	positive effect on the anaerobic digestion performance of the energy grass.
282	
283	Table 2.
284	
285	Figure. 4
286	
287	4. Conclusions
288	The height of <i>Pennisetum</i> hybrid at which it was harvested was demonstrated to
289	have significant effects on silage quality and the subsequent anaerobic digestion. The
290	results from silage quality of different materials concluded a linear relationship between
291	the stem and whole plant. Microbial community analysis revealed that Lactobacillus
292	was the dominant genus in stem silage, and reached the maximum at harvesting height
293	of 100 cm. This suggested that the stem had a primary influence on the silage quality.
294	The maximum specific methane yield was 356 ± 28 mL/g VS for the whole plant at a

14 / 25

height of 100 cm, indicating that a harvesting height of 100 cm could be the optimalfrom the perspective of silage quality and biogas production.

297

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307 **Reference**

- S. Mohapatra, C. Mishra, S. S. Behera, H. Thatoi, Application of Pretreatment,
 Fermentation and Molecular Techniques for Enhancing Bioethanol Production
 from Grass Biomass a Review, Renew Sust Energ Rev, 78 (2017), 1007-32.
- Y. Zhang, X. H. Kang, Z. M. Wang, X. Y. Kong, L. H. Li, Y. M. Sun, S. N. Zhu,
 S. R. Feng, X. J. Luo, P. M. Lv, Enhancement of the Energy Yield from Microalgae
 Via Enzymatic Pretreatment and Anaerobic Co-Digestion, Energy, 164 (2018),
 400-07.
- L.H. Li, Y.M. Sun, Z.H. Yuan, P.M. Lv, X.H Kang, Y. Zhang, G.X. Yang, Influence
 of the Feedstock Ratio and Organic Loading Rate on the Co-digestion Performance
 of Pennisetum hybrid and Cow Manure, Energ Fuel, 32(4) (2018) 5171-5180.
- S. Vanatpornratt, P. Nipon, Economic Feasible Evaluation of Biogas Production
 from Napier Grass, Res J Biotechnol, 10 (2015) 94-98.
- 5. C. Somerville, H. Youngs, C. Taylor, S.C. Davis, S.P. Long, Feedstocks for
 Lignocellulosic Biofuels, Science, 329 (2010) 790-792.
- K. Koch, Correlation between
 Biogas Yield and Chemical Composition of Grassland Plant Species, Energ Fuel,
 29 (2015) 7221-7229.
- 325 7. A. Elgersma, K. SøEgaard, Changes in nutritive value and herbage yield during

- extended growth intervals in grass-legume mixtures: effects of species, maturity at
 harvest, and relationships between productivity and components of feed quality.
 Grass Forage Sci (2017) 78-93.
- L.H. Li, Y.M. Sun, Z.H. Yuan, X.Y. Kong, Y. Wang, Influence of Harvest Period and Frequency on Methane Yield of Pennisetum Hybrids, J Energ Eng, 142 (2016).
- 9. D. Masse, Y. Gilbert, P. Savoie, G. Belanger, G. Parent, D. Babineau, Methane
 yield from switchgrass and reed canarygrass grown in Eastern Canada, Bioresource
 Technol, 102 (2011) 10286-10292.
- 10. D. Masse, Y. Gilbert, P. Savoie, G. Belanger, G. Parent, D. Babineau, Methane
 yield from switchgrass harvested at different stages of development in Eastern
 Canada, Bioresource Technol, 101 (2010) 9536-9541.
- 11. A. Lehtomaki, T.A. Viinikainen, J.A. Rintala, Screening boreal energy crops and
 crop residues for methane biofuel production, Biomass Bioenerg, 32 (2008) 541550.
- 12. F. Dragoni, V. Giannini, G. Ragaglini, E. Bonari, N. Silvestri, Effect of Harvest
 Time and Frequency on Biomass Quality and Biomethane Potential of Common
 Reed (Phragmites australis) Under Paludiculture Conditions, Bioenerg Res, 10
 (2017) 1066-1078.
- 13. R. Wahid, S.F. Nielsen, V.M. Hernandez, A.J. Ward, R. Gislum, U. Jorgensen, H.B.
 Moller, Methane production potential from Miscanthus sp.: Effect of harvesting
 time, genotypes and plant fractions, Biosyst Eng, 133 (2015) 71-80.
- 14. S. T Desta, X. J Yuan, L Junfeng, S Tao, Ensiling Characteristics, structural and
 nonstructural carbohydrate composition and Enzymatic Digestibility of Napier
 grass Ensiled with Additives, Bioresource Technol, 221 (2016) 447-454.
- 15. H. Wei, Y. Hu, S. Li, J. Huang, Q. Nie, H. Zhao, J. Tang, Simultaneous dark
 fermentative hydrogen and ethanol production from waste bread in a mixed packed
 tank reactor, J Clean Prod 141 (2017) 608-611.
- 16. L.H. Li, Y.M. Sun, Z.H. Yuan, X.Y. Kong, Y. Wao, L.G. Yang, Y. Zhang, D. Li,
 Effect of microalgae supplementation on the silage quality and anaerobic digestion
 performance of Manyflower silvergrass, Bioresource Technol, 189 (2015) 334-340.
- 17. C. Rodriguez, A. Alaswad, K. Y. Benyounis, A. G. Olabi, Pretreatment techniques
 used in biogas production from grass, Renew Suste Energ Rev 68(2) (2017) 11931204.
- 18. H. Wei, J. Fang, Z. Liu, J. Tang, Techno-economic evaluation of a combined
 bioprocess for fermentative hydrogen production from food waste, Bioresource
 Technol, 202 (2016) 107-112.
- 19. O. Pakarinen, A. Lehtomaki, S. Rissanen, J. Rintala, Storing energy crops for
 methane production: Effects of solids content and biological additive, Bioresource
 Technol, 99 (2008) 7074-7082.
- 20. H. Vervaeren, K. Hostyn, G. Ghekiere, B. Willems, Biological ensilage additives
 as pretreatment for maize to increase the biogas production, Renew Energ, 35
 (2010) 2089-2093.
- 21. L.H. Li, Z.H. Yuan, Y.M. Sun, X.Y. Kong, P.Y. Dong, J. Zhang, A reused method
 for molasses-processed wastewater: Effect on silage quality and anaerobic digestion

- performance of Pennisetum purpereum, Bioresource Technol, 241 (2017) 1003-1011.
- 22. D.D. Nguyen, S.W. Chang, S.Y. Jeong, J. Jeung, S. Kim, W.S. Guo, H.H. Ngo, Dry
 thermophilic semi-continuous anaerobic digestion of food waste: Performance
 evaluation, modified Gompertz model analysis, and energy balance, Energ Convers
 Manage, 128 (2016) 203-210.
- 376 23. K.C. Surendra, S.K. Khanal. Effects of crop maturity and size reduction on
 377 digestibility and methane yield of dedicated energy crop. Bioresource Technol. 178
 378 (2015) 187-93.
- 24. J.E. Erickson, K.R. Woodard, L.E. Sollenberger, Optimizing Sweet Sorghum
 Production for Biofuel in the Southeastern USA Through Nitrogen Fertilization and
 Top Removal, Bioenerg Res, 5 (2012) 86-94.
- 25. L.P. Han, Y. Steinberger, Y.L. Zhao, G.H. Xie, Accumulation and partitioning of
 nitrogen, phosphorus and potassium in different varieties of sweet sorghum, Field
 Crop Res, 120 (2011) 230-240.
- 385 26. J. Nousiainen, M. Rinne, M. Hellämäki, P. Huhtanen, Prediction of the digestibility
 386 of the primary growth of grass silages harvested at different stages of maturity from
 387 chemical composition and pepsin-cellulase solubility, Anim Feed Sc Tech 103(1)
 388 (2003) 97-111.
- 27. M. Rinne, Digestive processes of dairy cows fed silages harvested at four stages of
 grass maturity, J Anim Sci 80(7) (2002) 1986-1998.
- 28. P. Kalač, The required characteristics of ensiled crops used as a feedstock for biogas
 production: a review, Journal of Agrobiology, 28 (2011) 85-96.
- 29. M.M. Chen, Q.H. Liu, G.R. Xin, J.G. Zhang, Characteristics of lactic acid bacteria
 isolates and their inoculating effects on the silage fermentation at high temperature,
 Lett Appl Microbiol, 56 (2013) 71-78.
- 30. M.I. Petrova, E. Lievens, S. Malik, N. Imholz, S. Lebeer, Lactobacillus species as
 biomarkers and agents that can promote various aspects of vaginal health, Front
 Physiol, 6 (2015) 81-98.
- 31. F. Lebreton, A.L. Manson, J.T. Saavedra, T.J. Straub, A.M. Earl, M.S. Gilmore,
 Tracing the Enterococci from Paleozoic Origins to the Hospital, Cell, 169 (2017)
 849-861.
- 32. Y. Zhang, L. Li, X. Kong, F. Zhen, Z. Wang, Y. Sun, P. Dong, P. Lv, Inhibition
 Effect of Sodium Concentrations on the Anaerobic Digestion Performance of
 Sargassum Species, Energ Fuel, 31 (2017) 7101-7109.
- 33. Y. Zhang, M.A. Alam, X. Kong, Z. Wang, L. Li, Y. Sun, Z. Yuan, Effect of salinity
 on the microbial community and performance on anaerobic digestion of marine
 macroalgae, Journal of Chemical Technology & Biotechnology, 92 (2017) 23922399.
- 34. Z. Kimura, K.M. Chung, H. Itoh, A. Hiraishi, S. Okabe, Raoultella electrica sp nov.,
 isolated from anodic biofilms of a glucose-fed microbial fuel cell, Int J Syst Evol
 Micr, 64 (2014) 1384-1388.
- 35. S. Doijad, C. Imirzalioglu, Y.C. Yao, N.B. Pati, L. Falgenhauer, T. Hain, B.U.
 Foesel, B. Abt, J. Overmann, M.M. Mirambo, S.E. Mshana, T. Chakraborty,

- Enterobacter bugandensis sp nov., isolated from neonatal blood, Int J Syst Evol
 Micr, 66 (2016) 968-974.
- 36. V. Tilvikiene, Z. Kadziuliene, Z. Dabkevicius, K. Venslauskas, K. Navickas,
 Feasibility of tall fescue, cocksfoot and reed canary grass for anaerobic digestion:
 Analysis of productivity and energy potential, Ind Crop Prod, 84 (2016) 87-96.
- 37. C. Luca, R. Pilu, F. Tambone, B. Scaglia, F. Adani, New energy crop giant cane
 (Arundo donax L.) can substitute traditional energy crops increasing biogas yield
 and reducing costs, Bioresource Technol, 191 (2015) 197-204.
- 38. H.Z. Niu, X.Y. Kong, L.H. Li, Y.M. Sun, Z.H. Yuan, X.Y. Zhou, Analysis of
 Biogas Produced from Switchgrass by Anaerobic Digestion, Bioresources, 10
 (2015) 7178-7187.
- 39. E. Allen, D. M. Wall, C. Herrmann, J. D. Murphy, A detailed assessment of
 resource of biomethane from first, second and third generation substrates, Renew
 Energ 87 (2016) 656-665.

- 429 **Table captions:**
- **Table 1.** Characteristics of the fresh and silage materials of *Pennisetum* hybrid.
- 431 **Table 2.** Anaerobic digestion performance and kinetic parameters of the samples of
- 432 *Pennisetum* hybrid.
- 433 **Figure captions:**
- **Figure. 1.** The parameters determining the silage quality of different samples: (a) pH
- 435 values, (b) NH₃-N concentrations.
- 436 Figure. 2. The correlations of (a) pH values and (b) NH₃-N concentration between the
- 437 silages of stem, leaf, and whole-plant.
- **Figure. 3.** Bacterial compositions at the (a) phylum and (b) genus level of the samples
- 439 of *Pennisetum* hybrid. (Note: JX: Stem of fresh materials, YX: leaf of fresh materials,
- 440 HX: whole plant of fresh materials; JQ: stem of silage samples, YQ: leaf of silage
- 441 samples, HQ: whole plant of silage samples)
- 442 Figure. 4. Comparison of the cumulative biogas yields from the samples of
- 443 *Pennisetum* hybrid.
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			TS (%)	VS (%)	C (%)	N (%)	C/N
Fresh material	70cm	Whole	13.91±1.07	11.79±0.86	39.14±0.01	1.08±0.01	36.24±0.46
		Leaf	18.13±0.09	15.44±0.30	40.44±0.14	1.43±0.01	28.28±0.18
		Stem	11.97±0.57	10.51±.64	39.72±0.11	0.48±0.11	83.62±1.02
	100cm	Whole	14.58±0.53	12.56±0.28	39.89±0.11	0.88 ± 0.01	45.59±0.50
		Leaf	18.37±0.46	16.11±0.50	40.61±0.08	1.50±0.01	27.16±0.07
		Stem	11.92±0.49	10.56±0.61	39.83±0.10	0.51 ± 0.00	78.10±0.19
	150cm	Whole	23.11±1.65	20.29±2.34	40.98±0.09	1.03±0.01	39.79±0.46
		Leaf	25.73±1.08	22.04±1.33	41.03±0.11	1.35±0.01	30.55±0.08
		Stem	23.07±0.03	21.22±0.03	41.84±0.05	0.43±0.01	98.45±1.75
Silage material	70cm	Whole	15.09±0.52	12.34±0.29	40.72±0.06	0.98±0.02	41.77±0.97
		Leaf	18.82±0.25	15.77±0.48	40.83±0.22	1.34±0.01	30.58±0.00
		Stem	11.79±0.27	10.00±0.32	40.74±0.06	0.54 ± 0.01	75.46±2.09
	100cm	Whole	13.72±0.35	11.55±0.34	39.59±0.04	0.92±0.02	43.28±0.96
		Leaf	16.82±0.78	13.52±0.64	40.48±0.04	1.26±0.01	32.13±0.39
		Stem	10.58±0.71	9.00±0.60	41.12±0.16	0.54 ± 0.00	76.19±0.29
	150cm	Whole	22.05±0.86	18.81±0.86	41.28±0.05	0.64 ± 0.01	64.51±1.50
		Leaf	28.52±0.56	23.83±0.81	41.00±0.16	1.41±0.05	29.20±0.92
		Stem	18.32±3.31	16.04±3.47	41.79±0.05	0.36±0.01	117.73±2.21

Table 1. Characteristics of the fresh and silage materials of *Pennisetum* hybrid.

			Anaerobic digestion	Kinetic parameter			
Samples		performance (mL/g VS)	P R _m (mL/g VS) (mL/g VS d)		۸ (d)	R ²	
Fresh	70 cm	Whole	237.62	232.27	29.35	0.33	0.996
material		Leaf	240.90	235.87	29.87	0	0.995
		Stem	283.60	273.97	33.15	0	0.988
	100 cm	Whole	235.67	226.60	29.66	0	0.990
		Leaf	200.40	197.12	20.69	0.10	0.998
		Stem	232.85	224.00	30.38	0	0.988
	150 cm	Whole	226.37	219.68	23.15	0	0.984
		Leaf	258.34	249.32	31.28	0	0.987
		Stem	193.70	194.04	13.31	0	0.988
Silage	70 cm	Whole	270.04	267.75	47.23	0.57	0.999
material		Leaf	263.17	259.43	35.26	0.45	0.998
		Stem	298.04	293.52	46.45	0.34	0.997
	100 cm	Whole	355.77	350.56	43.41	0.39	0.997
		Leaf	315.75	312.90	40.74	0.82	0.999
		Stem	361.25	353.73	46.25	0.11	0.993
	150 cm	Whole	275.73	271.60	21.95	0	0.983
		Leaf	256.23	248.56	29.63	0	0.982
		Stem	277.11	271.72	23.13	0	0.981

Table 2. Anaerobic digestion performance and kinetic parameters of the samples of

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Pennisetum hybrid.

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Figure. 1. The parameters determining the silage quality of different samples: (a) pH

values, (b) NH₃-N concentrations.

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464 **Figure. 2.** The correlations of (a) pH values and (b) NH₃-N concentration between the

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HX: whole plant of fresh materials; JQ: stem of silage samples, YQ: leaf of silage 471

samples, HQ: whole plant of silage samples) 472

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474 **Figure. 4.** Comparison of the cumulative biogas yields from the samples of

Pennisetum hybrid