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Comparison of pre-treatments to reduce salinity and enhance biomethane yields of *Laminaria digitata* harvested in different seasons

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- 1 Comparison of pre-treatments to reduce salinity and enhance biomethane yields of
- 2 Laminaria digitata harvested in different seasons
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4

10 Abstract

- 11 Pre-treatment can enhance anaerobic digestion of seaweed; however, seasonal variation in
- 12 the biochemical composition of seaweed has a significant impact on the pre-treatment
- 13 effect. In this study, various pre-treatments were employed for the brown seaweed
- 14 Laminaria digitata harvested in March (with high ash content and low carbon to nitrogen
- 15 (C:N) ratio) and September (with low ash content and high C:N ratio). Washing of *L. digitata*
- 16 harvested in March with hot water (defined as 40 °C) removed 54% of the ash and improved
- 17 the volatile solids (VS) content by 31% leading to an improved biomethane yield of 282 L
- 18 CH₄ kg VS⁻¹. This pre-treatment affected a 16% increase in biodegradability, reduced salt
- 19 accumulation in the digestate by 54%, and increased specific methane yield per wet weight
- 20 by 25%. This level of effect was not noted for seaweed harvested in September, when the
- 21 biodegradability is higher.
- 22

23 Keywords: Laminaria digitata; Seaweed; Pre-treatment; Anaerobic digestion; Biomethane

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24 1. Introduction

Anaerobic digestion is a well-established technology with a potentially higher gross energy yield per hectare as compared to liquid biofuel land-based biomass systems; seaweed biomethane does not compete with food for arable land [1-6]. Brown seaweeds are reported as an abundant marine bioresource in Irish waters [7]. The feedstock received more attention after the European Parliament communication that advanced biofuels (such as from seaweed) should represent at least 1.25% of renewable energy supply in transport (RES-T) [8].

Brown seaweed harvested during different times round the year displays a significant 32 33 seasonal variation in biochemistry that has a significant influence on the digestibility of the seaweed for biogas production [4, 9, 10]. According to seasonal variation studies, autumn 34 35 was considered the best harvesting period for biogas production [11]. Biodegradability of 36 Laminaria digitata is lower in winter and spring; this can be attributed to lower levels of 37 readily digestible carbohydrates (such as laminarin and mannitol), higher ash contents (mostly salts) and a higher level of process inhibitors [9, 12]. Ash is the significant 38 39 component of brown seaweed that changes greatly through the whole year and can be up to 35% of dry weight [9, 13]. It has been suggested that due to high ash (salt) content in 40 Spring, the seaweed is not recommended for biogas production [4]. Accumulation of salts in 41 42 long-term digestion can be problematic [14]; this problem is heightened when the seaweed 43 is harvested in Winter or early Spring [4, 15]. However, if significant salt removal of the feedstock could be achieved (with effective low energy input pre-treatments), the possibility 44 of utilization of the Spring harvested biogas to produce biomethane could be closer to levels 45 achieved from the autumn harvested seaweed. To increase the digestibility and 46 47 degradability of brown seaweed (particularly in Spring), some pre-treatment may aid biogas

48 production; however, due to the absence of cellulose and lignin, harsh pre-treatment may
49 not be required [16].

50 The literature outlines pre-treatments employed on biomass such as physical (washing) [17], 51 mechanical (size reduction by cutting, chopping, beating and maceration) [18], chemical [19], hydrothermal (heating) [20] and thermochemical processes [17]. Mechanical pre-52 treatment is considered as the most suitable approach for seaweed biogas production [5, 53 54 21], whereas chemical pre-treatment was found to be inhibitory [17]. Beating pre-treatment was identified as an efficient method to enhance the specific methane yield [11]. The brown 55 56 seaweed L. digitata was found more suitable for beating pre-treatment to achieve a high 57 specific methane yield than Ascophyllum nodosum [22]. Nevertheless, mechanical pretreatments such as ball milling and beating have been described as high energy input pre-58 treatment methods for seaweed [23]. These methods involve multi-step processing (cutting, 59 60 drying, milling and sieving prior to processing) and include for installation of energy 61 intensive machinery [24]. Moreover, these mechanical pre-treatment methods do not lead to a reduction in salt accumulation in the bioreactor during digestion. It was revealed that 62 63 deionized water and acid pre-treatment could wash away the salt (ash), including sodium, potassium, magnesium, calcium and aluminium metal ions from the seaweed Enteromorpha 64 [25]. Therefore, energy-saving pre-treatment methods (such as hot washing of seaweed and 65 maceration) may be applied to brown seaweed to remove salts and other inhibitory 66 67 components to improve subsequent biogas production. 68 The authors suggest two significant gaps in the state of the art of seaweed biomethane:

assessment of effective low energy input pre-treatments to reduce the effect of salt

- 70 accumulation (and associated inhibition) in digestion; and the variation in the effect of these
- 71 pre-treatments on seaweeds harvested in different seasons. The innovation of the current

72	work is to highlight the effect of combined but simple low energy pre-treatment
73	methodologies (such as washing and maceration) on biomethane yields from L. digitata,
74	(the dominant brown seaweed in the Atlantic waters surrounding the UK and Ireland)
75	harvested in spring (when it is slow to biodegrade) and in autumn (when biodegradability is
76	highest). The objectives of this study are to:
77	• Examine the effect of pre-treatments on spring and autumn harvests of <i>L. digitata</i> ;
78	• Study the improvement in the biomethane yield and process dynamics;
79	• Investigate the effect of pre-treatment on salt accumulation in the batch reactor.
80	
81	2. Materials and Methods
82	2.1 Collection and processing of <i>L. digitata</i> for pre-treatments
83	L. digitata was collected from Roaring Water Bay, Co. Cork, in the south of Ireland (51°N, -
84	9°E) during March (spring in the northern hemisphere) and September (autumn in the
85	northern hemisphere). Combinations of various pre-treatments were applied to investigate
86	the effects on the biomethane yields based on seasonal variation in chemical composition.
87	Washing pre-treatment of the seaweed was carried out at two different temperatures,
88	namely: 15 \pm 1 °C described hereafter as cold water; and 40 \pm 1 °C described as hot water.
89	The fresh fronds (blades) of the seaweed were washed with cold water for 3 minutes to
90	remove any foreign particles. After washing, two mechanical pre-treatments (cutting and
91	maceration) were applied to reduce the particle size. The washed samples were cut by
92	scissors to a size of approximately 4 cm (hereafter termed CC: cold cut). Some samples were
93	subsequently were macerated in a Buffalo macerator to further reduce the size to less than
94	4 mm (hereafter termed CM: cold macerated).

95	The seaweed samples (fronds only) were washed with hot water for 3 minutes, and then cut
96	with scissors (termed HC: Hot cut) to allow comparison with CC. The samples washed with
97	hot water were macerated (termed HM: hot macerated) to the same size (4 mm) to
98	compare it with CM. Fresh unwashed samples were directly cut into a particle size of
99	approximately 4 cm and used as the control group (referred to as untreated). All samples
100	were frozen at -20 °C before analysis and before assessment for biomethane potential
101	(BMP).
102	
103	2.2 Analytical methods
104	Total solid (TS), volatile solid (VS) and ash were analysed by using the standard method of
105	drying of the second for 24 hours at 105 $^{\circ}$ C and subsequent compustion for 2 hours at 550

drying of the seaweed for 24 hours at 105 °C and subsequent combustion for 2 hours at 550

106 °C [26]. Elemental analysis was assessed by preparing the seaweed samples through drying

at 105 °C for 24 hours and then grinding to pass through a 500 μ m sieve. Dried samples

108 were analysed for carbon, hydrogen, nitrogen and oxygen (oxygen calculated by difference)

109 using a CE 440 elemental analyser.

110

111 2.3 Anaerobic digestion of the seaweed

The theoretical methane potential (TMP) was calculated by inserting the relative ratios of
carbon, hydrogen and oxygen in the seaweed composition into the Buswell equation (Eq.
(1)). The output from this equation provides a maximum potential methane yield [27]. The
molar volume of the gases was taken as 22.14 L at 0 °C and 1 atm.

117
$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2 O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) C H_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) C O_2$$
 Eq. (1)

The inoculum was sourced from lab-scale continuous stirred-tank reactors (operated at 37 118 119 °C), processing various substrates such as grass, dairy slurry and seaweed. The BMP tests of 120 the seaweed were conducted in a bioprocess system (Bioprocess AMPTS II[®] system). The 121 Bioprocess AMPTS system is an automated methane potential test system with output to a software package. The BMP system has the capacity to accommodate 15 glass bottles, 122 which served as batch digesters. Each glass bottle has a total volume of 650 ml with a 123 124 working volume of 400 ml. All glass bottles were sealed with rubber corks and were purged with nitrogen gas for five minutes to create an anoxic environment. The bottles contained a 125 126 continuous mixing system operating at 30 rpm and were kept at 37 °C using a water bath. 127 Carbon dioxide and hydrogen sulphide were removed by passing the gas through 3 M sodium hydroxide solution. The gas flow was measured by a gas tipping device and the 128 volume was automatically normalized to standard temperature (0 °C) and pressure (1 atm) 129 130 and zero moisture content by the Bioprocess AMPST II® system. The substrate to inoculum 131 ratio (S:I) on a VS basis, of 1:2 was used [28, 29]. To calculate the specific biomethane production, the total average biomethane produced by the inoculum was subtracted from 132 133 the average biomethane produced by each sample [30]. Batch trials were conducted in 134 triplicate, and the results were expressed as mean value \pm standard deviation. Salinity (g/L) and pH of the batch digestion processes were also recorded before and after each BMP 135 136 assay to investigate the effect of pre-treatment on the reaction performance and the gas 137 yield.

138

139 2.4 Process dynamics and statistical analysis

The study of the process dynamics is beneficial to facilitate an understanding of the changes
in the biodegradability and in the rate of biodegradability of the substrate before and after

142	pre-treatment. The kinetic parameters such as a change in the decay constant (days ⁻¹),
143	maximum yield (Y_{max}) and half-life (days) were obtained by taking data from the cumulative
144	methane production curves (after 30 days) and analysing in MATLAB software through a
145	first order differential equation as described previously [4, 31]. The biodegradability index
146	(BI) was defined as the ratio of the BMP yield to the theoretical value as expressed by the
147	TMP (from Eq. (1)).
148	Statistical significance of each pre-treatment was determined through the use of statistical
149	software (SPSS, IBM NY, USA). Analysis of variance (ANOVA) was performed to examine the
150	effect of various pre-treatments on different parameters (such as ash removal,
151	improvement in gas yields and enhancement in bio-degradability of the substrate). The
152	significance level was determined by multiple comparisons (Post Hoc test).
153	
154	3. Results and Discussion

155 3.1 Effect of pre-treatment on the seaweed composition

L. digitata was characterized for compositional and elemental analyses (Table 1). It should 156 be noted that the untreated March seaweed has a ratio of ash to volatile solids (A:V) of 0.51 157 compared to 0.24 for September and a C:N of 8.2 as compared to the September sample of 158 159 39.4. Ideally the C:N should be greater than 20 for optimal digestion performance [4, 6]. It may be stated that the untreated March seaweed is not as suitable for anaerobic digestion 160 as the September sample. March and September harvests of the seaweed were compared 161 before and after each pre-treatment (Table 1). Pre-treatment can remove the attached salts 162 of L. digitata, thereby decreasing the ash content leading to a change in the VS content 163 164 when expressed as a percentage of fresh weight. After pre-treatment, it was revealed that 165 washing with cold water did not remove a substantial amount of salts and hence did not

improve the VS composition of the substrate. However, the VS content of the seaweed 166 167 harvested in March was improved from 6.5% to 7.0% when washed with hot water and 168 macerated (HM) to a particle size of less than 4 mm (Table 1). For samples harvested in 169 March, HM pre-treatment succeeded in reducing ash content from 33.3% to 15.6%, resulting in increasing organic matter content from 66.7% to 84.4% and decreasing A:V ratio 170 from 0.51 to 0.19. The substantial removal of ash (salt) content should make the seaweed 171 172 more degradable [4, 12]. Significance of salt (ash) removal up to 54% in this study (Fig. 1) can be compared with previous studies in which pre-treatment of seaweed biomass with 173 174 deionized water and with acid was found helpful in salt removal [25]. Removal of ash and improvement of VS content for the March harvest can be advantageous for long-term 175 continuous digestion, as salt accumulation was reported as high in batch and continuous 176 177 digestion processes [4, 15, 16]. 178 However, for samples harvested in September, the VS content is relatively stable. This can

be attributed to the fact that the removal of salt may be accompanied by the removal of
soluble organic materials, such as mannitol, which is abundant in *L. digitata* harvested in
autumn [12].

The C:N ratio and the A:V ratio are considered as the key factors for digestion of seaweed 182 [4]. Washing with cold water of the March sample did not lead to a rise in the C:N ratio; 183 184 however, washing with hot water did. Hot water pre-treatment may cause the removal of 185 nitrogenous compounds such as proteins, lectins and alkaloids [32] and ultimately lead to an 186 improvement in the C:N ratio (from 8.2 to 13.8 in this study). Hot water pre-treatment also 187 facilitated the reduction in the A:V ratio from 0.51 to 0.19 in the March sample due to substantial removal of ash (Table 1). The percentage improvement of the C:N ratio was 188 greater in the March harvest than the September harvest due to the higher content of 189

190	nitrogenous compounds in the March seaweed than the September seaweed [4]. Removal
191	of ash, improvement of VS content and increase of the C:N ratio for the March harvest is
192	advantageous for long-term digestion, as salt accumulation was reported as significant in
193	batch and continuous digestion processes and potentially inhibitory to digestion at elevated
194	levels [4, 15]. The C:N ratio of the seaweeds in this current study were either lower (March)
195	or higher (September) than the reported optimum values (20 to 30), however, no acid
196	accumulation was observed during the digestion as evidenced from a buffered pH (ranged
197	from 7.0 to 7.7) after the 30-day trial.
198	
199	3.2 Impact of pre-treatment on the biogas yield
200	Seaweed harvested in spring (March) displayed a higher ash content and lower organic
201	matter content, which would significantly reduce biomethane yield [4]. To investigate the
202	effect of pre-treatment on the gas yield, various pre-treatments were designed and
203	compared. The BMP results revealed that size reduction (4 cm and 4 mm) after washing
204	with cold water had little effect on the specific methane yield expressed as L CH_4 kg VS ⁻¹ ,
205	when compared to the same size reduction after hot washing (Table 2).
206	It was observed (Table 2) that the effect of hot washing was more significant on the March
207	harvest (from 245 L CH $_4$ kg VS $^{-1}$ to 283 L CH $_4$ kg VS $^{-1}$) than the September harvest (from 280 L
208	$\rm CH_4$ kg VS ⁻¹ to 326 L CH ₄ kg VS ⁻¹). The rationale for this difference can be explained by the
209	difference in seasonal chemical composition. Seaweed harvested in March had high ash
210	content as compared to September (Table 1), hence, there is more significant potential for
211	ash removal [4].

Particle size reduction of dried seaweed was reported as an effective pre-treatment for
biogas production from brown seaweeds [33]; however, drying is considered to be an

214 energy intensive process on an industrial scale. In the current trials maceration to a particle 215 size of 4 mm is deemed unnecessary as compared to size reduction by scissors to 4 cm 216 particle size (Table 2). The gas yield was almost the same for both particle sizes for the 217 March harvest (282 L CH₄ kg VS⁻¹ and 283 L CH₄ kg VS⁻¹). However, the specific methane yield calculated based on wet weight highlights that maceration is an optimal step for the 218 March harvest (Table 2). The specific yield, improved by 25% (to 20 m³ CH₄t wwt⁻¹) for 219 220 maceration after hot washing, as compared to the sample cut by scissors (15 m³ CH₄ t wwt⁻¹) (Table 2). On the other hand, size reduction by scissors (4 cm) after hot washing decreased 221 the specific methane yield compared to the untreated sample (16 m³ CH₄ twwt⁻¹). This may 222 223 be attributed to material loss during manual cutting with scissors and can be avoided on an industrial level using mechanical instruments. 224

The current study can be correlated with other mechanical pre-treatments such as ball milling and beating [23]. However, these techniques involved multi-step processing (prior to the digestion) and require more intensive energy input [24]. Additionally, such mechanical pre-treatment methods have no impact on the issue of accumulation of salts in the bioreactor during digestion. The current pre-treatment method is unique in that it is simple (hot washing and subsequent maceration) but also successfully reduces the inhibitory effect of salt accumulation in bioreactors.

The exact mechanism or possible reason behind the hot washing pre-treatment is not fully known. However, it may be explained that removal of salts associated with the cell wall polysaccharide alginate (such as sodium, potassium, magnesium, etc.) along with some inhibitory components changed the biochemistry of the seaweed and made the substrate more degradable. This has also been reported in a previous study that described the effect of washing the seaweed biomass with deionized water [25]. Scanning electron microscopy

238	(SEM) confirmed that the seaweed biomass surfaces were eroded by removing salt (ash)
239	contents and extracts [25]. Other potential inhibitors, such as poly-phenols, sulphated
240	polysaccharides (fucoidan), fucoxanthin and associated epiphytes, can be removed via hot
241	washing [32]. It was reported that washing can also remove the epiphytes, which have high
242	antimicrobial activities, from the surface of the seaweed [32].
243	
244	3.3 Effect of pre-treatment on the key process parameters
245	Ash content, organic matter content and C:N ratio are the key process parameters, which
246	can affect biodegradability and the gas yield. Seaweed with lower ash content, higher
247	organic matter content, and C:N ratio in the optimum range are advantageous for biogas
248	production [4, 9].
249	Ash content in marine biomass can be an issue in long-term anaerobic digestion through
250	accumulation of salts in the digester. Tabassum et al., [4] charted the seasonal variation of L.
251	digitata through the twelve months of the year and found the salt build-up was higher in
252	winter and spring samples of seaweed as compared to summer and autumn samples. In this
253	work, hot water washing reduced the ash content by 54% (March) and 31% (September)
254	when cut to a particle size of 4 cm by scissors; values of 47% and 27% were achieved
255	respectively when macerated (Fig. 1). Ash removal resulted in an increased organic matter
256	content of 31% and 8% in March and September, respectively when the seaweed was cut by
257	scissors after hot water washing.
258	BI (defined as the ratio of the BMP yield to the theoretical yield) explains the process
259	efficiency in terms of degradability of the substrate in the reactor. BI improved from 0.52 to
260	0.61 of the seaweed harvest in March while it was increased from 0.62 to 0.76 for the
261	September harvest (Table 2). The substrate was 16% and 23% more biodegradable as
	A A

compared to untreated seaweed for March and September harvest, respectively (Fig. 1).
The higher BI in September as compared to the March harvest may be due to higher
concentrations of easily degradable organic matter content (such as mannitol) in the
substrate [9, 12].

Accumulation of salts was recorded before and after pre-treatments to examine the

reduction of salts in the reactor. Salinity and the A:V ratio were reported as key factors 267 268 affecting the gas yield during different harvesting seasons. Higher values of salinity and A:V led to lower values of biomethane production [4]. The salinity of seaweed was subtracted 269 270 from the salinity of inoculum (6.85 \pm 0.46 g/L) to calculate the salinity increase in the batch 271 digestion due to the seaweed only. It was observed that hot water pre-treatment successfully resulted in lowering the A:V ratio and salinity (Fig. 2) as compared to cold water 272 pre-treatment. However, the reduction in the percentage salinity was comparatively higher 273 274 for the March harvest (Fig. 2). A low biomethane yield was expected at a high salinity level 275 [34-36]. Application of hot water washing (hot cut) as a pre-treatment technology before anaerobic digestion of the seaweed resulted in 54% less salt accumulation in the reactor as 276 277 compared to the untreated March harvest.

278 After the process parameter studies, it was revealed that pre-treatment has the greatest

impact on the March harvest. While, for the September harvest, the impact of pre-

treatment is lower due to the already higher organic matter content, lower ash content and

lower levels of inhibitory compounds in the substrate as compared to the March harvest.

282

266

283 3.4 Process dynamics and statistical analysis

The changes in the process dynamics after each pre-treatment are listed in Table 3.

285 Maceration after hot water washing (HM) indicated significant kinetic decay increase as the

k value doubled from 0.10 to 0.20 for the March harvest and the biomass was degraded 286 efficiently (half-life was shortened from 6.8 to 3.4 days). This may be attributed to the 287 288 removal of a substantial amount of inhibitory compounds (such as polyphenols) from the 289 substrate that may be responsible for slower degradation of the seaweed in the untreated 290 sample [37]. The decay values as the result of other pre-treatments for the same harvest remained close to the untreated sample (Table 3). The decay constants of L. digitata were 291 292 comparable with those of perennial ryegrass, food waste, and brown seaweed reported previously in the range 0.11 to 0.19 [30, 38, 39]. After employing the HM pre-treatment for 293 294 the March sample, the decay constant was improved by 100%, whereas the half-life 295 methane production was reduced by 49%. The half-life of biomethane production (T_{50}) from untreated seaweed was reported as 4-5 296 days in summer and 6-9 days in winter [3] probably due to the higher concentration of easily 297 298 degradable laminarin and mannitol in autumn[9]. Improvement in kinetic parameters for 299 the March harvest (after pre-treatments) delimited the utilization of the substrate for biogas production and may reduce the retention time for digestion of the substrate to less 300 301 than 20 days, which is suggested sufficient for long-term continuous digestion [16, 30]. The experimental results were supported by statistical significance by conducting an ANOVA 302 analysis. Multiple comparisons results from one-way ANOVA indicated that pre-treatment 303 304 applied to the March harvest were significant (F=8.39, P < 0.001) as compared to the 305 September harvest (F=1.68, P < 0.23). The change or improvement in the specific methane 306 yield (of March harvest) was also found statistically significant (F=11.56, P < 0.001) while for 307 the seaweed harvested in the September harvest, the values were not statistically 308 significant (F=1.68, P < 0.23). The samples harvested in March were analysed further in 309 comparison with different factors affecting the methane yield. These factors were particle

310	size (4 mm and 4 cm) and washing method (hot water and cold water) in comparison with
311	the removal of salts (ash), biodegradability and ultimately the BMP enhancement. After
312	comparison, it was revealed that the most significant factor for the process was the A:V
313	ratio (F = 11.97 and P < 0.001) and the ash content (F = 14.09 and P < 0.001). Particle size
314	and washing method comparison results indicated that the most significant pre-treatment
315	was maceration to a particle size of 4 mm after hot washing (P < 0.002).
316	After comparison of statistical results, it can be concluded that maceration after hot
317	washing was the most efficient pre-treatment method to enhance the methane yield by
318	substantial removal of salts (ash) from the substrate. However, optimization of pre-
319	treatment time and temperature for seaweed is necessary to further improve the
320	performance of biogas production.
321	
322	4. Conclusions
323	Maceration of the brown seaweed after washing with cold water has little impact on the gas
324	yield as compared to washing with hot water. Hot washing pre-treatment resulted in higher
325	ash content removal from the March harvest than the September harvest. Scissor cutting

- 326 after hot washing yielded 16% higher biomethane by removing 54% ash with an
- 327 improvement of 31% of VS content. Maceration after hot washing pre-treatment

328 significantly achieved a 25% higher specific methane yield per unit wet weight compared to

- 329 the untreated sample. However, hot washing requires optimization of pre-treatment time
- and temperature to facilitate the continuous supply of the seaweed even in March for
- 331 biogas production.
- 332
- 333

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- 340

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449

450 Fig.1. Impact of various pre-treatment on the process parameters of *L. digitata*





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454 U untreated; C cold cut; CM cold macerated; HC hot cut; HM hot macerated

455 Fig. 2. Effect of pre-treatment on salts accumulation in the batch process.

Pre-treatment	Compositional Analysis				Elemental Analysis					
_	TS (%)	VS (%)	OMC (%)	Ash (%)	A:V	C %	Н%	N %	0 %	C:N
March										
Untreated	9.74 (0.02)	6.49 (0.14)	66.67	33.33 (1.37)	0.51	30.41 (0.90)	3.97 (0.11)	3.70 (0.06)	28.58	8.22
Cold cut	9.04 (0.20)	5.72 (0.20)	63.26	36.73 (0.50)	0.58	28.09 (0.38)	3.46 (0.07)	3.55 (0.34)	28.16	7.91
Hot cut	6.47 (0.06)	5.37 (0.09)	83.09	16.91 (0.06)	0.20	39.29 (0.12)	4.83 (0.05)	2.84 (0.05)	36.12	13.83
Cold macerated	9.76 (0.12)	6.56 (0.07)	67.19	32.81 (0.05)	0.49	30.41 (0.90)	3.97 (0.11)	3.70 (0.57)	29.10	8.22
Hot macerated	8.32 (0.10)	7.02 (0.02)	84.38	15.62 (0.7)	0.19	39.53 (0.01)	4.88 (0.12)	2.91 (0.29)	37.06	13.58
September										
Untreated	19.46 (0.26)	15.67 (0.25)	80.51	19.49 (0.44)	0.24	36.62 (0.17)	5.30 (0.05)	0.93 (0.03)	39.37	39.38
Cold cut	19.44 (0.34)	15.60 (0.35)	80.27	19.43 (0.43)	0.24	36.74 (0.17)	5.03 (0.11)	1.18 (0.08)	37.32	31.14
Hot cut	15.51 (0.36)	13.42 (0.32)	86.56	13.44 (0.30)	0.16	38.98 (0.17)	5.21 (0.11)	0.97 (0.25)	41.39	40.19
Cold macerated	19.46 (0.26)	15.67 (0.25)	80.51	19.49 (0.44)	0.24	36.62 (0.17)	5.30 (0.05)	0.93 (0.03)	37.66	39.38
Hot macerated	16.82 (0.10)	14.42 (0.10)	85.75	14.25 (0.14)	0.17	38.20 (0.07)	5.39 (0.02)	0.93 (0.10)	41.23	41.08

457 Table 1 Change in the chemistry of *L. digitata* after different pre-treatments

TS is total solids, VS is volatile solids, OMC is organic matter content obtained dividing VS/TS, A:V is ash to volatile solid ratio, while C, H, N, O and C:N are carbon, hydrogen,
 nitrogen, oxygen and carbon to nitrogen ratio, respectively. Standard deviation is in parentheses

460

461

Pre-treatment	BMP yield	ТМР	BI	Specific yield	
	(L CH ₄ kg VS ⁻¹)	(L CH ₄ kg VS ⁻¹)	(BMP/TMP)	(m ³ CH ₄ t wwt ⁻¹)	
March					
Untreated	245 (10.86)	469	0.52	16	
Cold cut	258 (12.42)	469	0.55	15	
Hot cut	283 (6.24)	468	0.60	15	
Cold macerated	265 (3.56)	469	0.57	17	
Hot macerated	282 (2.33)	462	0.61	20	
September					
Untreated	280 (28.76)	450	0.62	44	
Cold cut	303 (22.55)	450	0.67	47	
Hot cut	326 (26.25)	424	0.76	44	
Cold macerated	307 (18.98)	450	0.68	48	
Hot macerated	308 (5.63)	403	0.76	44	

462 Table 2 Effect of pre-treatments on the gas yield and specific yield production

463 BMP, TMP, BI and wwt are biomethane potential, theoretical methane potential, biodegradability and wet

464 weight, respectively. Standard deviation is in parentheses.

3

465 Sample calculation for specific methane yield:

466 Specific yield (March, un-treated) = $0.245 \text{ m}^3 \text{ CH}_4 \text{ t VS}^{-1} \times 64.9 \text{ (kg VS per t wwt, (Table 1))} = 16 \text{ m}^3 \text{ CH}_4 \text{ t wwt}^{-1}$

Pre-treatment	K (days ⁻¹)	R ²	Y _{max}	<i>T₅₀</i> (days)
March				
Untreated	0.10	0.98	245	6.81
Cold cut	0.08	0.97	258	8.89
Hot cut	0.08	0.96	283	8.43
Cold macerated	0.10	0.97	265	6.81
Hot macerated	0.20	0.99	282	3.46
September				
Untreated	0.13	0.95	282	5.24
Cold cut	0.15	0.99	338	4.68
Hot cut	0.08	0.95	326	8.20
Cold macerated	0.13	0.96	307	5.24
Hot macerated	0.09	0.93	308	7.49
Cellulose	0.17	0.99	356	4.09

468 Table 3 The process dynamics of *L. digitata* based on different pre-treatments

k is the decay constant, R^2 is a measure that how the kinetic model fits the biomethane potential curve (%),

 Y_{max} is the maximum methane potential and T_{50} is the half-life methane production (days).

- Maceration after hot washing of *L. digitata* significantly affected biogas yield.
- Impact of the pre-treatment was more significant on March harvest than September.
- The pre-treatment removed 54% of ash whilst increasing VS content by 31%.
- Bio-digestibility was enhanced by 16% with 54% less salt accumulation in digester.
- Specific methane yield of *L. digitata* per weight wet was increased by 25%.

A CERTING