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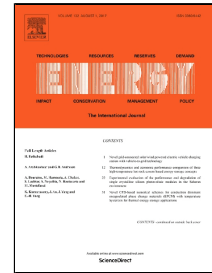
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Comparison of pre-treatments to reduce salinity and enhance biomethane yields of *Laminaria digitata* harvested in different seasons

Muhammad Rizwan Tabassum, Ao Xia, Jerry D. Murphy



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

3 Muhammad Rizwan Tabassum ^a, Ao Xia ^{b*}, and Jerry D. Murphy ^{a, c}

4
5 a. MaREI Centre, Environmental Research Institute, University College Cork, Cork, Ireland

6 b. Key Laboratory of Low-grade Energy Utilization Technologies and Systems, Chongqing
7 University, Chongqing 400044, China

8 c. School of Engineering, University College Cork, Cork, Ireland

9

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

* Corresponding author at: Key Laboratory of Low-grade Energy Utilization Technologies and Systems, Chongqing University, Chongqing 400044, China. E-mail address: aoxia@cqu.edu.cn (Ao Xia).

24 1. Introduction

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

32 Brown seaweed harvested during different times round the year displays a significant
33 seasonal variation in biochemistry that has a significant influence on the digestibility of the
34 seaweed for biogas production [4, 9, 10]. According to seasonal variation studies, autumn
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
38 (mostly salts) and a higher level of process inhibitors [9, 12]. Ash is the significant
39 component of brown seaweed that changes greatly through the whole year and can be up
40 to 35% of dry weight [9, 13]. It has been suggested that due to high ash (salt) content in
41 Spring, the seaweed is not recommended for biogas production [4]. Accumulation of salts in
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
44 feedstock could be achieved (with effective low energy input pre-treatments), the possibility
45 of utilization of the Spring harvested biogas to produce biomethane could be closer to levels
46 achieved from the autumn harvested seaweed. To increase the digestibility and
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
52 [19], hydrothermal (heating) [20] and thermochemical processes [17]. Mechanical pre-
53 treatment is considered as the most suitable approach for seaweed biogas production [5,
54 21], whereas chemical pre-treatment was found to be inhibitory [17]. Beating pre-treatment
55 was identified as an efficient method to enhance the specific methane yield [11]. The brown
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 pre-
58 treatments such as ball milling and beating have been described as high energy input pre-
59 treatment methods for seaweed [23]. These methods involve multi-step processing (cutting,
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
62 to a reduction in salt accumulation in the bioreactor during digestion. It was revealed that
63 deionized water and acid pre-treatment could wash away the salt (ash), including sodium,
64 potassium, magnesium, calcium and aluminium metal ions from the seaweed *Enteromorpha*
65 [25]. Therefore, energy-saving pre-treatment methods (such as hot washing of seaweed and
66 maceration) may be applied to brown seaweed to remove salts and other inhibitory
67 components to improve subsequent biogas production.

68 The authors suggest two significant gaps in the state of the art of seaweed biomethane:
69 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 *L. digitata*;
- 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 *L. digitata* 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 ± 1 °C described hereafter as cold water; and 40 ± 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

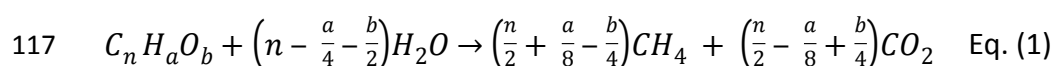
104 Total solid (TS), volatile solid (VS) and ash were analysed by using the standard method of
105 drying of the seaweed for 24 hours at 105 °C and subsequent combustion for 2 hours at 550
106 °C [26]. Elemental analysis was assessed by preparing the seaweed samples through drying
107 at 105 °C for 24 hours and then grinding to pass through a 500 µm 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

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

116



118 The inoculum was sourced from lab-scale continuous stirred-tank reactors (operated at 37
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
122 software package. The BMP system has the capacity to accommodate 15 glass bottles,
123 which served as batch digesters. Each glass bottle has a total volume of 650 ml with a
124 working volume of 400 ml. All glass bottles were sealed with rubber corks and were purged
125 with nitrogen gas for five minutes to create an anoxic environment. The bottles contained a
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
128 sodium hydroxide solution. The gas flow was measured by a gas tipping device and the
129 volume was automatically normalized to standard temperature (0 °C) and pressure (1 atm)
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
132 production, the total average biomethane produced by the inoculum was subtracted from
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)
135 and pH of the batch digestion processes were also recorded before and after each BMP
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

140 The study of the process dynamics is beneficial to facilitate an understanding of the changes
141 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

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

166 improve the VS composition of the substrate. However, the VS content of the seaweed
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%,
170 resulting in increasing organic matter content from 66.7% to 84.4% and decreasing A:V ratio
171 from 0.51 to 0.19. The substantial removal of ash (salt) content should make the seaweed
172 more degradable [4, 12]. Significance of salt (ash) removal up to 54% in this study (Fig. 1)
173 can be compared with previous studies in which pre-treatment of seaweed biomass with
174 deionized water and with acid was found helpful in salt removal [25]. Removal of ash and
175 improvement of VS content for the March harvest can be advantageous for long-term
176 continuous digestion, as salt accumulation was reported as high in batch and continuous
177 digestion processes [4, 15, 16].

178 However, for samples harvested in September, the VS content is relatively stable. This can
179 be attributed to the fact that the removal of salt may be accompanied by the removal of
180 soluble organic materials, such as mannitol, which is abundant in *L. digitata* harvested in
181 autumn [12].

182 The C:N ratio and the A:V ratio are considered as the key factors for digestion of seaweed
183 [4]. Washing with cold water of the March sample did not lead to a rise in the C:N ratio;
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
188 substantial removal of ash (Table 1). The percentage improvement of the C:N ratio was
189 greater in the March harvest than the September harvest due to the higher content of

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 $CH_4 kg VS^{-1}$ 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].

212 Particle size reduction of dried seaweed was reported as an effective pre-treatment for
213 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
218 yield calculated based on wet weight highlights that maceration is an optimal step for the
219 March harvest (Table 2). The specific yield, improved by 25% (to 20 m³ CH₄t wwt⁻¹) for
220 maceration after hot washing, as compared to the sample cut by scissors (15 m³ CH₄ t wwt⁻¹)
221 (Table 2). On the other hand, size reduction by scissors (4 cm) after hot washing decreased
222 the specific methane yield compared to the untreated sample (16 m³ CH₄ twwt⁻¹). This may
223 be attributed to material loss during manual cutting with scissors and can be avoided on an
224 industrial level using mechanical instruments.

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

232 The exact mechanism or possible reason behind the hot washing pre-treatment is not fully
233 known. However, it may be explained that removal of salts associated with the cell wall
234 polysaccharide alginate (such as sodium, potassium, magnesium, etc.) along with some
235 inhibitory components changed the biochemistry of the seaweed and made the substrate
236 more degradable. This has also been reported in a previous study that described the effect
237 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

262 compared to untreated seaweed for March and September harvest, respectively (Fig. 1).

263 The higher BI in September as compared to the March harvest may be due to higher

264 concentrations of easily degradable organic matter content (such as mannitol) in the

265 substrate [9, 12].

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

267 reduction of salts in the reactor. Salinity and the A:V ratio were reported as key factors

268 affecting the gas yield during different harvesting seasons. Higher values of salinity and A:V

269 led to lower values of biomethane production [4]. The salinity of seaweed was subtracted

270 from the salinity of inoculum (6.85 ± 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

272 successfully resulted in lowering the A:V ratio and salinity (Fig. 2) as compared to cold water

273 pre-treatment. However, the reduction in the percentage salinity was comparatively higher

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

276 anaerobic digestion of the seaweed resulted in 54% less salt accumulation in the reactor as

277 compared to the untreated March harvest.

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

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

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

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

282

283 3.4 Process dynamics and statistical analysis

284 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

286 k value doubled from 0.10 to 0.20 for the March harvest and the biomass was degraded
287 efficiently (half-life was shortened from 6.8 to 3.4 days). This may be attributed to the
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
291 remained close to the untreated sample (Table 3). The decay constants of *L. digitata* were
292 comparable with those of perennial ryegrass, food waste, and brown seaweed reported
293 previously in the range 0.11 to 0.19 [30, 38, 39]. After employing the HM pre-treatment for
294 the March sample, the decay constant was improved by 100%, whereas the half-life
295 methane production was reduced by 49%.

296 The half-life of biomethane production (T_{50}) from untreated seaweed was reported as 4-5
297 days in summer and 6-9 days in winter [3] probably due to the higher concentration of easily
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
300 biogas production and may reduce the retention time for digestion of the substrate to less
301 than 20 days, which is suggested sufficient for long-term continuous digestion [16, 30].

302 The experimental results were supported by statistical significance by conducting an ANOVA
303 analysis. Multiple comparisons results from one-way ANOVA indicated that pre-treatment
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
330 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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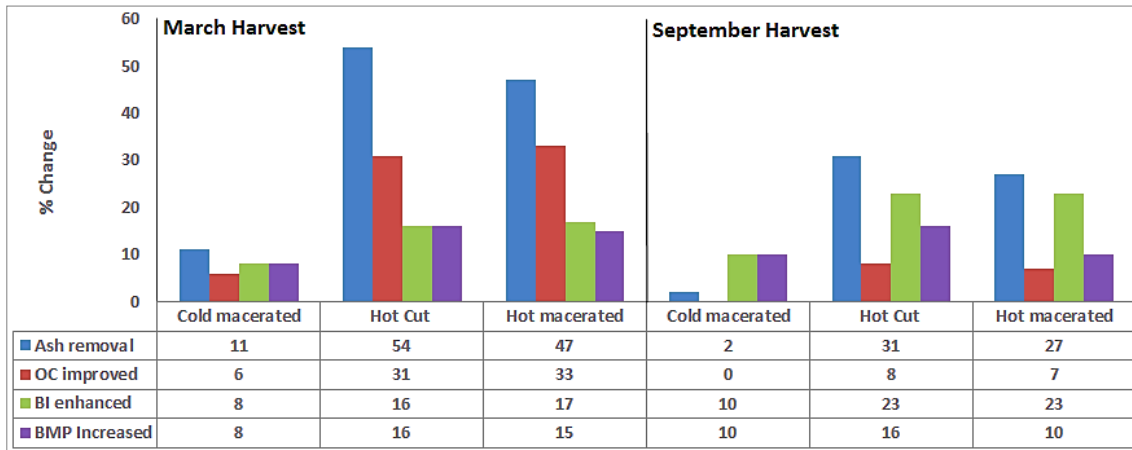
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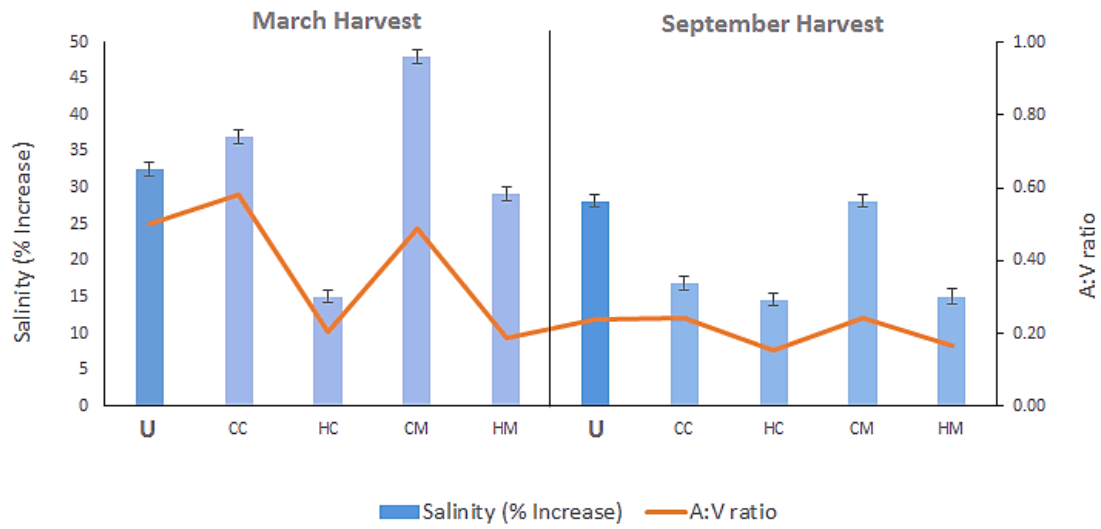


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450 Fig.1. Impact of various pre-treatment on the process parameters of *L. digitata*

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453

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.

456

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

Pre-treatment	Compositional Analysis					Elemental Analysis				
	TS (%)	VS (%)	OMC (%)	Ash (%)	A:V	C %	H %	N %	O %	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

458 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,
459 nitrogen, oxygen and carbon to nitrogen ratio, respectively. Standard deviation is in parentheses

460

461

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

Pre-treatment	BMP yield (L CH ₄ kg VS ⁻¹)	TMP (L CH ₄ kg VS ⁻¹)	BI (BMP/TMP)	Specific yield (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

463 BMP, TMP, BI and wwt are biomethane potential, theoretical methane potential, biodegradability and wet
 464 weight, respectively. Standard deviation is in parentheses.

465 Sample calculation for specific methane yield:

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

467

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

Pre-treatment	K (days ⁻¹)	R^2	Y_{max}	T_{50} (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

469 k is the decay constant, R^2 is a measure that how the kinetic model fits the biomethane potential curve (%),
 470 Y_{max} is the maximum methane potential and T_{50} is the half-life methane production (days).

471

- 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%.