

Please cite the Published Version

Tedesco, S and Daniels, Stephen (2019) Evaluation of inoculum acclimatation and biochemical seasonal variation for the production of renewable gaseous fuel from biorefined *Laminaria* sp. waste streams. *Renewable Energy*, 139. ISSN 1879-0682

DOI: <https://doi.org/10.1016/j.renene.2019.02.057>

Publisher: Elsevier

Version: Accepted Version

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1 **Evaluation of inoculum acclimation and biochemical seasonal variation for the**
2 **production of renewable gaseous fuel from biorefined *Laminaria* sp. waste**
3 **streams.**

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Declarations of interest: none.

14 **Abstract**

15 *Laminaria. sp.* seaweeds have been recognised the potential to greatly contribute to the
16 generation of renewable gaseous fuel via anaerobic digestion. Seaweed feedstock has been
17 documented to consistently vary its biochemical composition with seasons, which affects
18 stability of biomethane production. As currently seaweeds are too costly for use as third
19 generation feedstock for biofuels, this paper investigates the biogas potential of the algal
20 waste streams from the existing bio-industry. Analytical tests identified an improved
21 digestibility of extracted residues (C:N>20). Fermentation with and without inoculum
22 acclimation revealed the interaction between compositional seasonality and inoculum type
23 to significantly affect methane production from the extracted samples. Summer's
24 composition has the most significant impact on methane production, with best results
25 achieved with acclimatised inoculum (433 ml CH₄ gVS⁻¹ and final biodegradation of about
26 90%). Organics concentration (tCOD) and ash:volatile (A:V) ratio also play a major role in
27 the bioconversion process. In particular, digestion with acclimatised inoculum better responds
28 to A:V fluctuations across seasons, which produced the highest average methane yield of 334
29 ml gVS⁻¹. Pretreatments are required to increase the biodegradation index in spring and
30 summer when not using acclimation.

Acronyms: AD (Anaerobic Digestion), AS (Sludge, acclimatised), A:V (Ash to Volatile ratio) BI (Biodegradability Index), COD (Chemical Oxygen Demand), OMC (Organic Matter Content), S (Sludge, non-acclimatised), TS (Total Solids), VS (Volatile Solids).

31 *Keywords: Laminaria hyperborea, Seasonal variation, Integrated Biorefinery, Methane*
32 *Potential, Acclimatation, Anaerobic digestion.*

33

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37

38 **1. Introduction:**

39 In the recent years, there has been an ever-growing effort to generate biomass-derived fuels
40 in the attempt to mitigate the effects related to depletion of fossil fuels and climate
41 change/global warming. This has particularly increased interest for the development of a
42 future macroalgae biorefinery concept. Unlike first and second biofuel feedstock, macroalgae
43 (seaweeds) do not occupy arable land or water for growth [1] and are not quite used as food
44 source in western countries. Also, sugars depolymerisation is eased by negligible amounts of
45 compounds recalcitrant to energy conversion, such as hemicellulose and lignin [2, 3]. In
46 addition, faster growing rates [4] and higher carbon fixation capability [5] are among the
47 main benefits characterising marine biomass.

48 Despite holding an estimated gross energy contribution potential in the range of 38–384 GJ
49 $\text{ha}^{-1} \text{yr}^{-1}$ [6], the high cost of seaweed feedstock [7] currently makes its energy conversion
50 not economically viable. However, it has been identified that macroalgae are very promising
51 as potential biorefinery substrates [8], which leads to the need to investigate the challenges
52 for an optimal integrated biorefinery configuration. Since the development of integrated
53 biorefinery and bioenergy technologies is still at its infancy stage, retrofitting consolidated
54 bioconversion strategies, such as anaerobic digestion (AD), into existing facilities (especially
55 low-tech) will be key in addressing energy requirements locally in the immediate future.
56 Within this circular approach, AD of algal waste and residues from an extraction cascade
57 could find a fast and economic application to generate renewable gaseous fuel to be used to
58 satisfy energy requirements from internal processes. It has also been reported [9] that the
59 selection or integration of biorefinery technologies should be based on its waste
60 characterisation.

61 The literature lacks of investigations examining the biogas potential of the algal waste
62 streams from the existing bio-industry. Ireland's seaweed-based industry consists of small
63 and medium businesses involved in production of animal nutrition, animal hygiene, plant
64 health, soil fertilizers, alginate, cosmetics and nutraceutical products [10]. The Irish Fishery
65 Board (BIM), the Irish seaweed production and processing industry will be worth €30 million
66 per annum by 2020 [10]. When processed for extraction of bioproducts, a significant amount
67 of sugar-rich seaweed residues is generated [11] and this creates an opportunity for biogas
68 production.

69 A very recent review study has identified lack of knowledge of the characterisation and
70 biomethane potential of selected seaweeds as the first bottleneck to a seaweed-based biogas
71 industry [6]. The latter depend on both macroalgal species and change in composition due to
72 season variation. A number of studies [12-14] have investigated the effect of biochemical
73 seasonal variation of brown macroalgae. In particular, *Laminaria* sp. have been identified as
74 the most promising in terms of fermentable carbohydrates content [12, 15-16] for AD
75 applications. There is however insufficient knowledge about compositional variation of
76 *Laminaria hyperborea* (LH) for biogas production as well as lack of assessments of
77 biomethane potential from residues following extraction of common industrial bioproducts
78 such as alginic acid, fucoidan, fucoxanthin, laminarin, mannitol, and proteins. The innovation
79 of this paper is in the assessment the seasonal variation in composition for freshly harvested
80 and bioproducts-extracted biomass of *L. hyperborea*. Simultaneously, the effect of inoculum
81 acclimatation was investigated targeting a more efficient and maximised biomethane
82 production. The objectives of the research are:

- 83 • Investigate the biochemical seasonal variation of *L. hyperborea* biomass prior to and
84 after extraction of high-value bioproducts following an integrated biorefinery
85 approach.
- 86 • Assess how the seasonal variation affects the biomethane production of biorefined *L.*
87 *hyperborea* residues.
- 88 • Undertake a statistical analysis of biomethane potential essays to identify the benefits
89 of inoculum acclimatation over seasonal biodegradability rates across the year.

90

91 **2. Materials and Methods:**

92 2.1. Macroalgae biomass and inoculum

93 Biomass samples of *Laminaria hyperborea* (LH) were collected seasonally across a year
94 period (2015-16) in Howth, Co. Dublin, Ireland and then frozen to -20°C until use. The
95 collections started in May/June 2015 and were completed the following year. The results are
96 reported in relation to seasons as follows: spring (March 2016), summer (June 2015), autumn
97 (September 2015) and winter (December 2015). These then underwent bioproducts extraction
98 at room temperature at laboratory scale as per procedure provided by an Irish seaweed
99 company, Irish Seaweed Processors Ltd.

100 The extracted samples were incubated with 300 g of digested sewage sludge, provided by the
101 wastewater treatment plant of Celtic Anglian Water (CAW) Ltd. The initial sludge's pH in
102 was measured as 8.1 ± 0.02 . The digested sewage sludge was utilised to provide the required
103 micro-organisms to the digesters and was added as received and then after acclimatation in
104 two separate fermentation assays. Through each of the four seasonal experiments, only the
105 dry matter was characterised for the inoculum. Values ranged between 4.0% and 5.8% of dry
106 matter, with an average value of 4.8%. The sludge's acclimatation was conducted by
107 inoculating reactors with extracted *L. hyperborea*, allowing fermentation to occur for
108 approximately 10 days. After this period, the acclimatised sludge was filtered through a sieve
109 to remove any undigested seaweed solids and used as inoculum for a new digestion cycle.

110

111 2.2. Proximate and ultimate analysis

112 Total Solids (TS) and Volatile Solids (VS) in of the un-extracted and extracted samples were
113 characterised by using a high-temperature oven via overnight drying at 105 °C followed by
114 combustion at 575°C, as by standard procedure [17]. All tests were conducted in duplicate.

115 The ultimate analysis was outsourced to Celignis Ltd. (Irish biomass laboratory) to identify
116 the elemental composition of the fresh and residue substrates. The carbon, hydrogen,
117 nitrogen, and sulphur contents of samples were obtained according to the European Standard
118 procedure EN 15104:2011 [18], using an Elementar Vario MACRO Cube elemental analyser.

119 The oxygen content was calculated by difference according to the formula below:

120

$$121 \text{Oxygen (\%)} = 100 - \text{Carbon(\% Dry Basis)} - \text{Hydrogen(\% Dry Basis)} - \text{Nitrogen(\% Dry} \\ 122 \text{Basis)} - \text{Sulphur(\% Dry Basis)} - \text{Ash(\% Dry Basis)} \quad (\text{eq. 1})$$

123

124 2.3. Ambient extraction methodologies

125 *L. hyperborea*'s fronds were manually chopped down to roughly <0.5cm, sealed in a food
126 plastic bag containing about 200 g of chopped fronds. The bags were then extensively
127 perforated to maximise soaking in the reagent solution and kept below solvent level by the
128 aid of a weight. Room temperature was selected as it has been reported to be almost as
129 effective as high-temperature extractions [19], thus constituting a cheaper alternative for
130 seaweed processors to obtain bio-products. The procedures aim to extraction of pigments,
131 laminarin, mannitol and alginate. To simulate the industrial scale extraction process, the
132 biomass species were extracted in series in three steps using three separate buckets. These

133 contained respectively 3L of ethanol 99.9% pure for the first step, then a mild acid (acetic
134 acid pH 5.5) as second extraction and finally a 5L solution of 10% w/w Na₂CO₃ (pH 9.5).
135 After extraction was performed, samples were then manually squeezed for about a minute.
136 Subsequently, part of the samples were dried at 105±2 °C overnight in a muffle furnace and
137 then cooled down and stored in a desiccator until use for the proximate analysis, as described
138 in section 2.2. The remaining samples in the bag were instead prepared for organics
139 quantification and pH adjustment as described in section 2.4, in order to be used in the batch
140 AD trials.

141

142 2.4 pH adjustments and dissolved organics in leachates

143 Following ambient extraction, the pH of the samples was measured before and after digestion
144 using a Hanna precision pH meter, model pH 213. This was required as pH of the residues
145 was found above 9 following the alkaline extraction. Such pH value is not suitable for a
146 stable digestion process, which has been found to be 7.5 – 8.5 [20, 21]. Adjustments were
147 carried out with 0.1N sulphuric acid solution until pH reached neutral values (6.99-7.03).
148 Total COD (tCOD) is widely used to evaluate the amount of organic matter within water and
149 wastewater. This parameter was used in the study to estimate the organic matter dissolved in
150 the residue samples. This was accomplished by collection and analysis of the seaweed
151 leachates after the last extraction step, according to procedure provided by Hach Lange [22].
152 A Hach Lange DR2000 spectrometer was used for reading the tCOD values.

153

154 2.5. Set-up methods for batch experiments

155 The bioreactors set-up was conducted following procedure VDI 4630 [23]. The reactors
156 consisted of borosilicate glass flasks of 500 ml each in capacity. Each bioreactor was filled
157 with 300 g of inoculum (digested sewage sludge ‘S’ or acclimatised sludge ‘AS’) and 20 g of
158 seaweed residues, with an inoculum-to-substrate ratio of 15:1 on a wet weight basis. Each
159 bioreactor condition was performed in triplicate. The pH for each sample was adjusted with
160 0.1N sulphuric acid solution prior to incubation with the inoculum. A biogas analyser, model
161 Dräger X-Am 3000, was used to verify anaerobic conditions were created correctly when
162 preparing the reactors and to analyse the gas composition at the end of the collection period.
163 An upturned measuring cylinder was utilized to derive the dry biogas volume and the

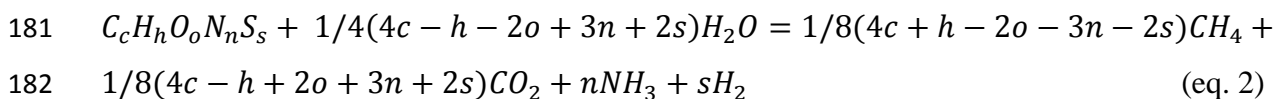
164 methane yields are reported for a gas in standard conditions (temperature of 0 °C and pressure
165 of 1 atm). The biogas volume in the collection bag was measured by water displacement in
166 the upturned measuring cylinder. Prior to biogas volume measurements, the system was
167 flushed with nitrogen to ensure no oxygen was present for subsequent biogas composition
168 analysis. After the nitrogen purge, the initial volume in the headspace of the cylinder
169 (nitrogen only) was recorded and then subtracted to the total measured biogas volume.

170 Water-baths were used to keep the reactors at a fixed mesophilic temperature of 38 ± 1 °C for
171 the duration of a retention time of 21 days. A control sample of each inocula in double
172 replication was used to determine the inoculum contribution to the biogas formation, which
173 has been then subtracted from the biogas digestion volume in order to determine the actual
174 yields of the seaweed residues.

175

176 2.6 Stoichiometric yields and anaerobic biodegradability

177 Buswell equation [24] (eq. 2) was used to derive the stoichiometric methane potential (SMP)
178 using the results from the elemental analysis described in section 2.2 before and after the
179 chemical extractions. The obtained SMP yields identify the maximum theoretical biomethane
180 potential that can be achieved from the substrate.



183 A biodegradability index (BI) was used to estimate the digestion efficiency via biochemical
184 methane potential (BMP) assays.

185 From eq. 2, the biodegradability index has been calculated as the ratio of the actual methane
186 yield to the stoichiometric methane yield.

187

188

189 2.7 Statistical analysis

190 Analysis of variance (ANOVA) [25] was used to investigate the effect on the methane yield
191 (BMP) of seasonal variation in biochemical composition and inoculum type, using Excel and
192 Design Expert (v.11).

193 In particular, two-factor ANOVA in Design Expert was conducted on the variable ‘season’ to
 194 investigate the impact of the substrates’ composition on BMP when digesting with a specific
 195 inoculum type. This also allowed to identify the effects on the interaction of compositional
 196 seasonal variation and inoculum type on BMP. This included a Least Significance Difference
 197 (LSD)-test with a $t(\frac{\alpha}{2}, N-a)$ as Post Hoc comparison method to assess which season has a
 198 major influence on methane production.

199

200

201 **3. Results and discussion:**

202 3.1 Composition variation of fresh and extracted feedstock on methane potential

203 *L. hyperborea* samples were characterised for proximate and ultimate analyses prior to
 204 chemicals extraction (Table 1). TS content ranged from 18% to about 29% with a peak in
 205 autumn for which the highest VS content was also found. The VS is also reported as % of TS,
 206 denominated as organic matter content (OMC). From Table 1, the highest TS and VS content
 207 were observed in September (29% and 24% wet weight basis respectively), which appears to
 208 be the best harvest period for *L. hyperborea*. Furthermore, the A:V ratio is the lowest in that
 209 period (0.17), which is advantageous for biomass degradation and suggests avoidance of
 210 sodium inhibition [14]. The ash fraction was high in summer (0.48), while OMC was found at
 211 its minimum (68%). Results from the proximate analysis indicate that VS content is generally
 212 in line with seasonal values identified for brown seaweeds [26].

213 The C:N ratio was found to oscillate between 8 and 21 approximately. This is not in range
 214 with the ideal values identified for anaerobic digestion of seaweed (>20) [27]. Highest values
 215 of C:N were recorded in the summer and autumn, during which carbohydrates accumulation
 216 should lead to suitable biodegradation rates. However, low C:N values in the cold months
 217 suggest *L. hyperborea* not to be suitable for AD mono-digestion, but another carbon-rich
 218 substrate to be added for adequate co-digestion to take place.

219

220 *Table 1 Seasonal characterisation of L. hyperborea*

Month of harvest	Proximate analysis					Ultimate analysis					
	TS %	VS %	OMC % (of TS)	Ash % (of TS)	A:V	C%	H%	N%	S%	O%	C:N

Spring	22.5 (0.26)	18.0 (0.04)	80.18	19.82 (0.04)	0.25	37.37 (0.09)	4.77 (0.01)	4.89 (0.03)	1.11 (0.12)	32.05 (0.07)	7.64
Summer	18.0 (0.20)	12.2 (0.13)	67.58	32.42 (0.06)	0.48	35.43 (0.08)	4.42 (0.05)	1.85 (0.04)	1.10 (0.08)	24.78 (0.06)	19.2
Autumn	28.5 (0.08)	24.4 (0.16)	85.67	14.33 (0.12)	0.17	39.65 (0.04)	5.25 (0.02)	1.93 (0.02)	1.40 (0.07)	37.45 (0.07)	20.6
Winter	17.4 (0.15)	13.2 (0.31)	76.41	23.59 (0.25)	0.31	34.94 (0.29)	5.74 (0.10)	2.48 (0.02)	0.75 (0.04)	32.50 (0.45)	14.1

Abbreviations: TS=Total Solids; VS=Volatile Solids; OMC=Organic Matter Content; A:V=Ash-to-Volatile ratio

221

222 *Table 2 Seasonal characterisation of L. hyperborea residues*

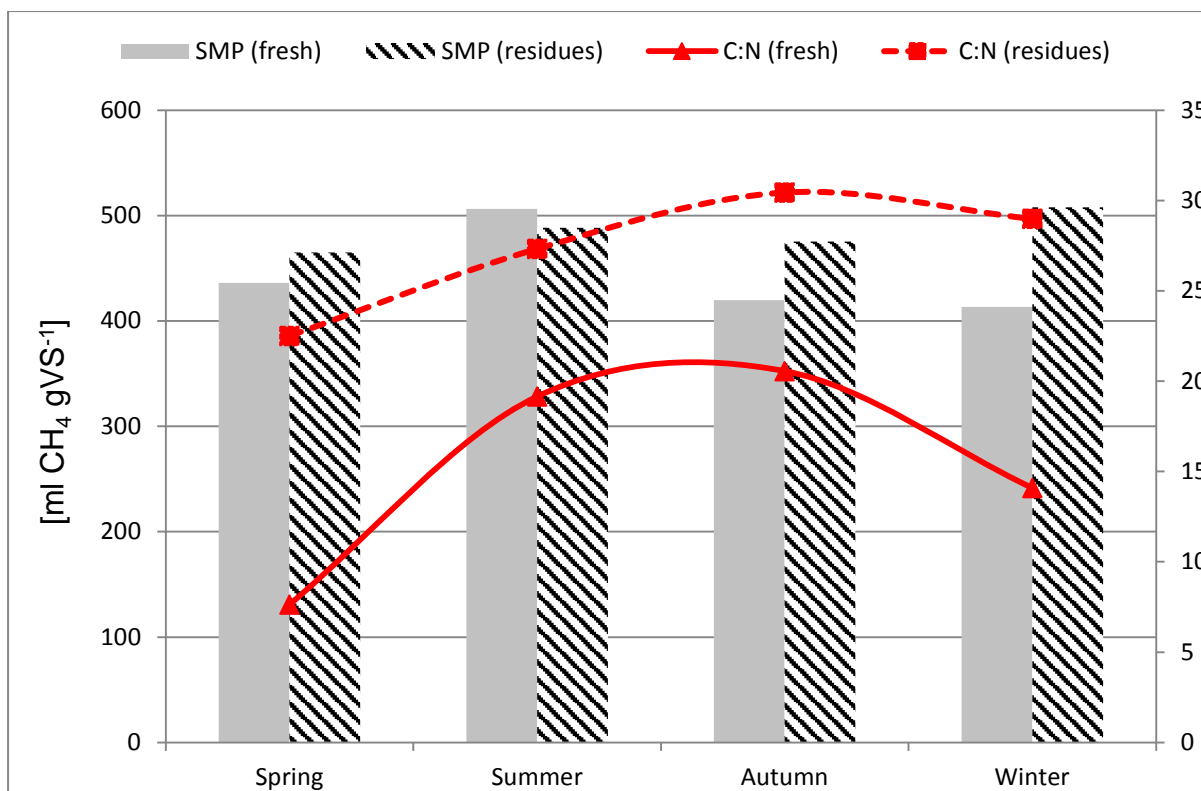
Month of harvest	Proximate analysis					Ultimate analysis					
	TS %	VS %	OMC % (of TS)	Ash % (of TS)	A:V	C%	H%	N%	S%	O%	C:N
Spring	23.5 (0.28)	19.4 (0.15)	82.47	17.53 (0.05)	0.21	41.30 (0.10)	4.92 (0.03)	1.83 (0.00)	1.05 (0.13)	33.36 (0.26)	22.5
Summer	16.0 (0.31)	11.3 (0.07)	70.62	29.38 (0.08)	0.42	36.62 (0.07)	4.1 (0.02)	1.34 (0.02)	0.38 (0.11)	28.18 (0.21)	27.3
Autumn	27.4 (0.27)	22.5 (0.02)	82.09	17.91 (0.04)	0.22	41.18 (0.08)	5.18 (0.00)	1.35 (0.01)	1.16 (0.39)	33.22 (0.30)	30.5
Winter	26.2 (0.01)	21.6 (0.30)	82.45	17.55 (0.25)	0.21	40.60 (0.03)	6.18 (0.05)	1.40 (0.00)	0.42 (0.06)	33.86 (0.05)	29.0

Abbreviations: TS=Total Solids; VS=Volatile Solids; OMC=Organic Matter Content; A:V=Ash-to-Volatile ratio

223

224 *L. hyperborea* residues were characterised for proximate and ultimate analyses (Table 2). TS
 225 content ranged from 16% to about 26% with a peak in autumn again which retained the
 226 highest VS content of almost 23%. The A:V ratio is relatively stable at 0.2 approximately in
 227 spring, autumn and winter however, it reaches its maximum in the summer with a value of
 228 0.42. The ash fraction during this period is the highest as well (about 29%) with VS content
 229 at its minimum. As per fresh stock, autumnal stock is expected to yield more methane as the
 230 A:V ratio is the lowest in that period, which is beneficial for conversion of biomass via AD
 231 [14].

232 The C:N ratio of the extracted samples was always within the ideal range for AD (20-30
 233 [28]), indicating LH residues can be used in mono-digestion systems. Overall it can be noted
 234 that extraction of bioproducts has improved the anaerobic digestibility in terms of C:N, see
 235 Figure 1, by far in some instances; i.e. spring and winter. This seems related to partial
 236 migration and/or retention of organics from the reagent solutions within the plant's structure.
 237 A similar but more pronounced behaviour was noticed in a parallel work were other species
 238 of brown seaweed underwent the same pretreatments/extractions [29]. The extent of C:N
 239 changes depends on season, harvest location and seaweed species.



240

241 *Figure 1 Stoichiometric methane potential (SMP) of fresh and extracted LH samples*

242

243 The SMP values calculated using eq. 2 are also reported in Figure 1. Theoretical values from
 244 the residues are mostly higher than those found in the fresh feedstock by 7%-22%, due to a
 245 fundamental change in elemental composition caused by the ambient extractions. Volumes of
 246 the SMP from extracted LH samples are in line with [30], which was conducted in the
 247 autumn season. Such high theoretical potentials indicate the suitability of LH's biorefined
 248 residues for methane production.

249

250 3.2 BMP assays and effect of inoculum acclimatation

251

252 From the two-way ANOVA in Table 3, it can be observed that the model is significant and
 253 the following conclusions can be extrapolated from the analysis: (i) the means for S and AS
 254 are different, (ii) the means for each season are different and (iii) the interaction
 255 season/inoculum type has a very significant effect on methane production. Also, this is
 256 particularly confirmed for the AB-interaction as p-value is very small (0.0002). Results
 257 indicate that the summer season has a very high impact on BMP from S inoculum, while
 258 spring, summer and autumn are more determinant when using AS. Fit statistics indicate the
 259 Predicted R² of 0.6846 is in reasonable agreement with the Adjusted R² of 0.8521 as their

260 difference is less than 0.2 and the model significance is confirmed by the value of adequate
 261 precision (>4) [25].

262 *Table 3 Two-way ANOVA for seasonal variation and inoculum type*

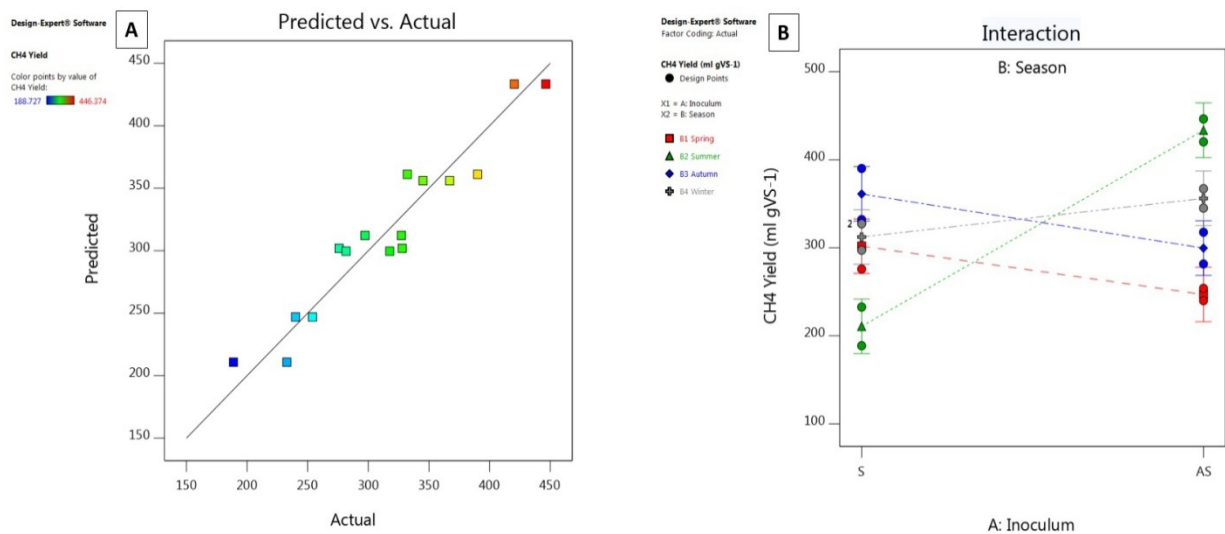
Source of Variation	SS	df	MS	F	P-value	F crit	
Model	67494.89	7	9642.12	13.35	0.0008	5.32	<i>significant</i>
A-Inoculum	5629.98	1	5629.99	7.79	0.0235	4.07	
B-Season	9202.77	3	3067.59	4.25	0.0452	4.07	
AB	52662.12	3	17554.04	24.31	0.0002		
Pure Error	5778	8	722.25				
Cor Total	73272.89	15					

$R^2 = 0.9211$; $Adj. R^2 = 0.8521$; $Pred. R^2 = 0.6846$; Adeq. Precision = 11.72.

263

264 Figure 2 (a) shows the model follows normal distribution of predicted versus actual values of
 265 methane yield in ml CH₄ gVS⁻¹ from the BMP assay, while Figure 2 (b) illustrates the
 266 interaction Season/Inoculum type (b) detected in the model by the 2-way ANOVA analysis.
 267 From the interaction plot, it can be noted summer's composition has the most significant
 268 impact on methane production, with best results achieved with acclimatation of inoculum
 269 (AS). Spring and autumn present a similar trend of methane production with slightly better
 270 yields (<100 ml CH₄ gVS⁻¹ difference) obtained in the autumn months using non-acclimatised
 271 inoculum (S). In winter the best yields are achieved with acclimatised inoculum with about
 272 350 ml CH₄ gVS⁻¹.

273



274

275 *Figure 2 Predicted vs Actuals normal distribution (a) and Interaction plot (b) from the two-way ANOVA*

276 Table 4 and Figure 3 summarise the results obtained by the BMP assays in terms of methane
 277 yields and overall biodegradation of the LH substrates in relation to the theoretical achievable
 278 yields (BMP/SMP). The best methane production occurs in the summer season using AS with
 279 a value of about 433 ml CH₄ gVS⁻¹ and very high final biodegradation (0.9). This yield is
 280 about 24.5% higher than volumes achieved from fresh *L. digitata* [16], which is currently
 281 considered the most promising for AD among brown species. Overall, by looking at the
 282 %CH₄ in the biogas from the SMP and the actual BMP values, it can be observed the results
 283 from the BMP assay confirm such digestion yields are far better than mono-digestion [31].
 284 These are higher on average +11.3% when using S and +9.5% when using AS when
 285 compared to the stoichiometric methane achievable. Highest average bioconversion rates in
 286 BI are instead obtained when using acclimatised (0.69) over non-acclimatised (0.61)
 287 inoculum, with a lead of +8% corresponding to 334 ml CH₄ gVS⁻¹. This result is higher than
 288 values obtained by [32] on fresh LH digestion however, the BMP was conducted in
 289 winter/spring, whose values are closer to those identified in this study. Results from Table 4
 290 are in line with findings of [12, 33, 34]. Benefits of inoculum acclimatation at stabilising
 291 biomethane production rates from seaweed digestion have also been found by [35, 36].

292 *Table 4 Biodegradability indices and methane yield in relation to inoculum type*

Month of harvest	S					AS		
	SMP [ml CH ₄ gVS ⁻¹]	CH ₄ %	BMP [ml CH ₄ gVS ⁻¹]	Cumulative CH ₄ %	BI (BMP/SMP)	BMP [ml CH ₄ gVS ⁻¹]	Cumulative CH ₄ %	BI (BMP/SMP)
Spring	465	51	301.9 (26)	64	0.65	246.9 (7)	60	0.53
Summer	488	51	210.7 (22)	60	0.43	433.4 (13)	62	0.89
Autumn	476	52	361.1 (29)	69	0.76	299.6 (18)	63	0.63
Winter	508	56	312.3 (15)	62	0.61	356.1 (11)	63	0.70

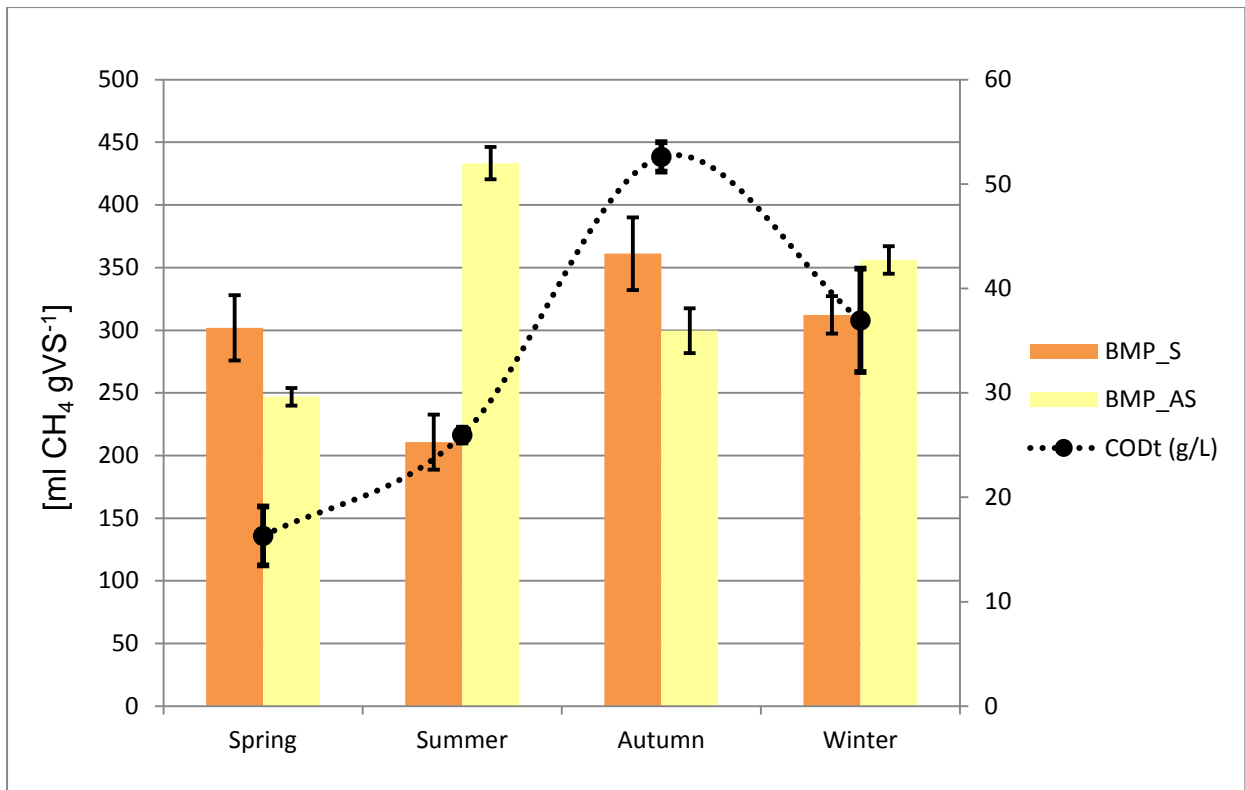
293

294 The lowest bioconversion rates can be identified in the summer when using S (0.43) and in
 295 the spring when using AS (0.53). In the first case, this result appears related to the A:V ratio
 296 which is the highest (0.42) reached by the substrate across the year, see Figure 4 (a), while
 297 acclimatation allows to overcome this obstacle for improved as well as maximised methane
 298 production. A similar behaviour of methane yielded in relation to A:V variations was
 299 observed by [14] on brown seaweed *Ascophyllum nodosum*. In addition, the sample is also
 300 characterised by relatively low tCOD, Figure 4 (b), which translates into less organics freely
 301 available to be hydrolysed by sugar-reducing bacteria. The latter reason is also behind the

302 lowest methane yield obtained when using AS in spring, as tCOD is at its lowest. This affects
303 significantly LH's bioconversion despite A:V being at its minimum. The adoption of a
304 pretreatment in these particular instances is expected to improve tCOD concentrations, even
305 by far [37-39], and therefore would be recommendable.

306 The highest methane production rates were expected in autumn due to the highest values of
307 tCOD combined with a very low A:V ratio in the substrate. Resulting methane yields in this
308 period were still high (in the range of 299.6-361.1 ml CH₄ gVS⁻¹) with BI as high as 0.76.
309 However, a yield lower than expectable can be justified by an overload in tCOD (53 g ml⁻¹)
310 which is believed to have caused an inhibition of the methanogenesis phase. It is worth
311 reporting that salt accumulation in this specific period has been found to inhibit methane
312 production from digestion of fresh seaweed [14]. However, this should not be the case for the
313 residues due to low A:V ratio detected (see Figure 4(a)).

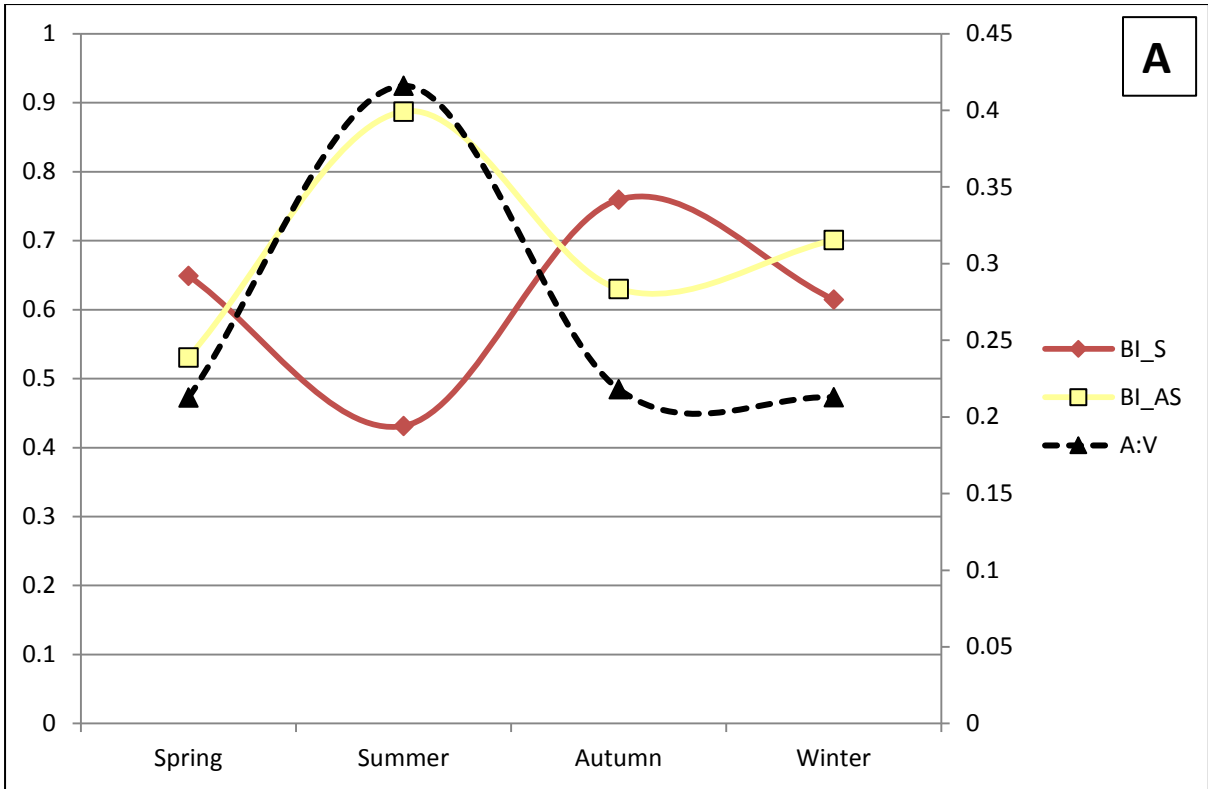
314 As it can be observed in Figure 4 (a), the highest A:V value has been recorded in the summer,
315 meaning more inorganic matter is present in the reactor. This led to a better performance with
316 AS and it was expected that acclimatation would be beneficial when ash content varies so
317 steeply from one season to the next. Differences in BIs in spring and autumn (refer to Figure
318 3) are within the order of about 50 mL gVS⁻¹. As know, the reason behind each seasonal
319 performance of the digester is also related to the kinetics of the metabolic bacterial activity in
320 addition to feedstock's biochemical properties and site of harvesting. In addition, seaweed
321 harvest is currently subjected to license by Government bodies and is kept down to a
322 minimum and to specific seasons (depending on local authorities) in order to avoid damage to
323 the marine ecosystems. This means that the supply is also unstable and will require a
324 carefully planned reactor design depending on both biochemical composition of the feedstock
325 and amount available to digest throughout the year.



326

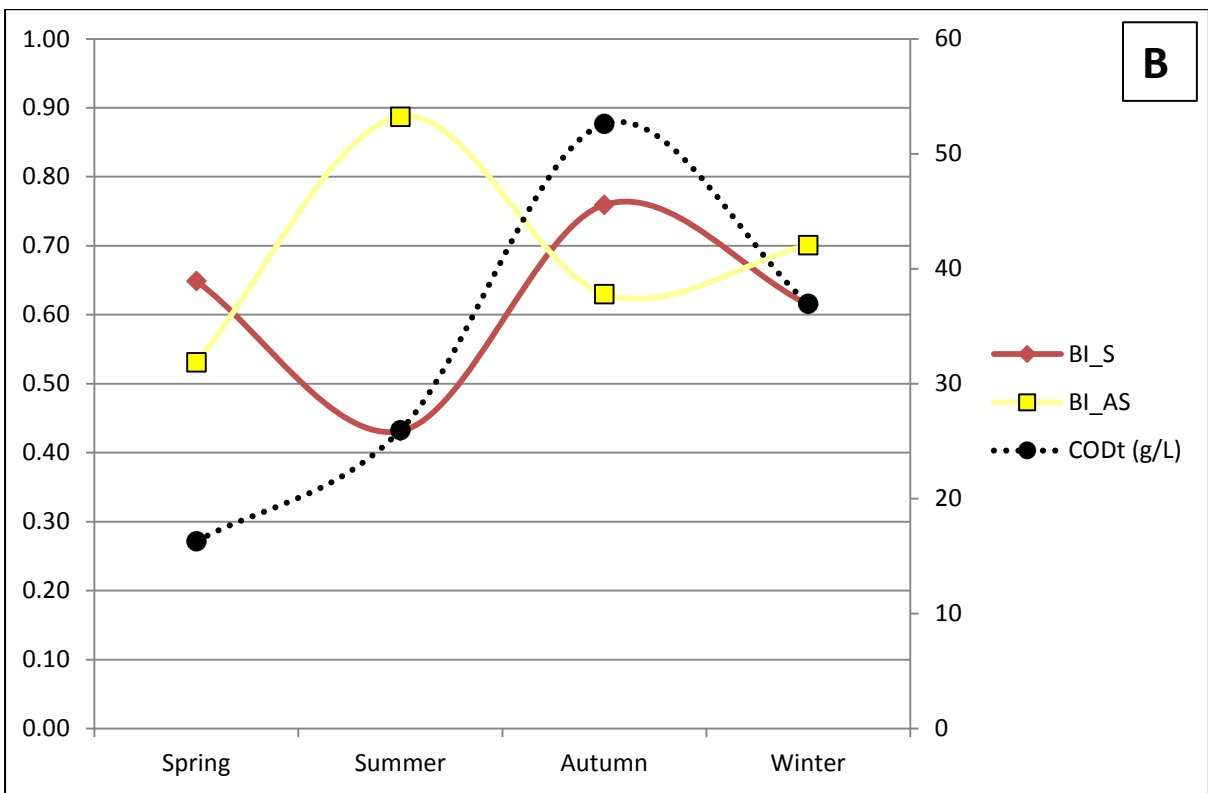
327 *Figure 3 BMP assay of L. hyperborea with and without inoculum acclimatation*

328



329

330



331

332 *Figure 4 Seasonality of BI indices from L. hyperborea's biodegradation against (a) A:V ratio and (b) tCOD concentration*

333

334 **4. Conclusion:**

335 This research aimed to determine the effect of seasonal variation in composition and
336 inoculum acclimatation for the anaerobic digestion of brown seaweed *L. hyperborea* residues
337 after ambient extraction cascade of a variety of bioproducts. The best methane production
338 occurs in the summer season using acclimatised sludge with a value of about 433 ml CH₄
339 gVS⁻¹ and a bioconversion rate of 0.9. Methane yields from the BMP assays are higher on
340 average +11.3% when using non-acclimatised sludge and +9.5% when using acclimatised if
341 compared to the stoichiometric methane that can be achieved from the substrates.

342 Inoculum acclimatation as well as biochemical seasonal variation has been found to
343 significantly affect the methane yields and to produce an interacting effect. An inhibitory
344 value of tCOD has been found at tCOD (53 g ml⁻¹) and A:V of 0.42. Methane production is
345 more stable at responding to A:V fluctuations if using acclimatised inoculum and produced
346 the highest average BI (0.69), with a highest average methane yield of 334 ml gVS⁻¹.

347

348 **Acknowledgements**

349 This research was supported by Science Foundation Ireland via the Technology and
350 Innovation Development Award (14/TIDA/2420). The authors would like to acknowledge
351 Irish Seaweed Processors (ISP Ltd.) for providing the procedure to extract the seaweed
352 biomass utilized in this research and Celtic Anglian Water (CAW) Ltd. for providing the
353 inoculum used in the actual fermentation trials. Additionally, the authors would like to thank
354 Dr Joseph Stokes for providing support in terms of facilities in the Mechanical and
355 Manufacturing Engineering Department to carry out this project.

356

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