# Evaluation of inoculum acclimatation and biochemical seasonal variation for the production of renewable gaseous fuel from biorefined *Laminaria* sp. waste streams.

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## Abstract

*Laminaria. sp.* seaweeds have been recognised the potential to greatly contribute to the generation of renewable gaseous fuel via anaerobic digestion. Seaweed feedstock has been documented to consistently vary its biochemical composition with seasons, which affects stability of biomethane production. As currently seaweeds are too costly for use as third generation feedstock for biofuels, this paper investigates the biogas potential of the algal waste streams from the existing bio-industry. Analytical tests identified an improved digestibility of extracted residues (C:N>20). Fermentation with and without inoculum acclimatation revealed the interaction between compositional seasonality and inoculum type to significantly affect methane production from the extracted samples. Summer's composition has the most significant impact on methane production, with best results achieved with acclimatised inoculum (433 ml $CH_4$ $gVS^{-1}$ and final biodegradation of about 90%). Organics concentration (tCOD) and ash:volatile (A:V) ratio also play a major role in the bioconversion process. In particular, digestion with acclimatised inoculum better responds to A:V fluctuations across seasons, which produced the highest average methane yield of 334 ml $gVS^{-1}$. Pretreatments are required to increase the biodegradation index in spring and summer when not using acclimatation.

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).

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

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## **1. Introduction**:

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

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

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

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

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

## 2. Materials and Methods:

### 2.1. Macroalgae biomass and inoculum

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

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

### 2.2. Proximate and ultimate analysis

Total Solids (TS) and Volatile Solids (VS) in of the un-extracted and extracted samples were characterised by using a high-temperature oven via overnight drying at 105 °C followed by combustion at 575°C, as by standard procedure [17]. All tests were conducted in duplicate.
The ultimate analysis was outsourced to Celignis Ltd. (Irish biomass laboratory) to identify the elemental composition of the fresh and residue substrates. The carbon, hydrogen, nitrogen, and sulphur contents of samples were obtained according to the European Standard procedure EN 15104:2011 [18], using an Elementar Vario MACRO Cube elemental analyser. The oxygen content was calculated by difference according to the formula below:

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

### 2.3. Ambient extraction methodologies

*L. hyperborea*'s fronds were manually chopped down to roughly <0.5cm, sealed in a food plastic bag containing about 200 g of chopped fronds. The bags were then extensively perforated to maximise soaking in the reagent solution and kept below solvent level by the aid of a weight. Room temperature was selected as it has been reported to be almost as effective as high-temperature extractions [19], thus constituting a cheaper alternative for seaweed processors to obtain bio-products. The procedures aim to extraction of pigments, laminarin, mannitol and alginate. To simulate the industrial scale extraction process, the biomass species were extracted in series in three steps using three separate buckets. These contained respectively 3L of ethanol 99.9% pure for the first step, then a mild acid (acetic acid pH 5.5) as second extraction and finally a 5L solution of 10% w/w $Na_2CO_3$ (pH 9.5). After extraction was performed, samples were then manually squeezed for about a minute. Subsequently, part of the samples were dried at 105±2 °C overnight in a muffle furnace and then cooled down and stored in a desiccator until use for the proximate analysis, as described in section 2.2. The remaining samples in the bag were instead prepared for organics quantification and pH adjustment as described in section 2.4, in order to be used in the batch AD trials.

## 2.4 pH adjustments and dissolved organics in leachates

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

## 2.5. Set-up methods for batch experiments

The bioreactors set-up was conducted following procedure VDI 4630 [23]. The reactors consisted of borosilicate glass flasks of 500 ml each in capacity. Each bioreactor was filled with 300 g of inoculum (digested sewage sludge ‘S’ or acclimatised sludge ‘AS’) and 20 g of seaweed residues, with an inoculum-to-substrate ratio of 15:1 on a wet weight basis. Each bioreactor condition was performed in triplicate. The pH for each sample was adjusted with 0.1N sulphuric acid solution prior to incubation with the inoculum. A biogas analyser, model Drager X-Am 3000, was used to verify anaerobic conditions were created correctly when preparing the reactors and to analyse the gas composition at the end of the collection period. An upturned measuring cylinder was utilized to derive the dry biogas volume and the methane yields are reported for a gas in standard conditions (temperature of 0 °C and pressure of 1 atm). The biogas volume in the collection bag was measured by water displacement in the upturned measuring cylinder. Prior to biogas volume measurements, the system was flushed with nitrogen to ensure no oxygen was present for subsequent biogas composition analysis. After the nitrogen purge, the initial volume in the headspace of the cylinder (nitrogen only) was recorded and then subtracted to the total measured biogas volume.

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

## 2.6 Stoichiometric yields and anaerobic biodegradability

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

$$C_cH_hO_oN_nS_s + 1/4(4c - h - 2o + 3n + 2s)H_2O = 1/8(4c + h - 2o - 3n - 2s)CH_4 + 1/8(4c - h + 2o + 3n + 2s)CO_2 + nNH_3 + sH_2 \quad \text{(eq. 2)}$$

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

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

## 2.7 Statistical analysis

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

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

## <u>**3. Results and discussion**</u>:

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

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

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

*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

*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

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

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

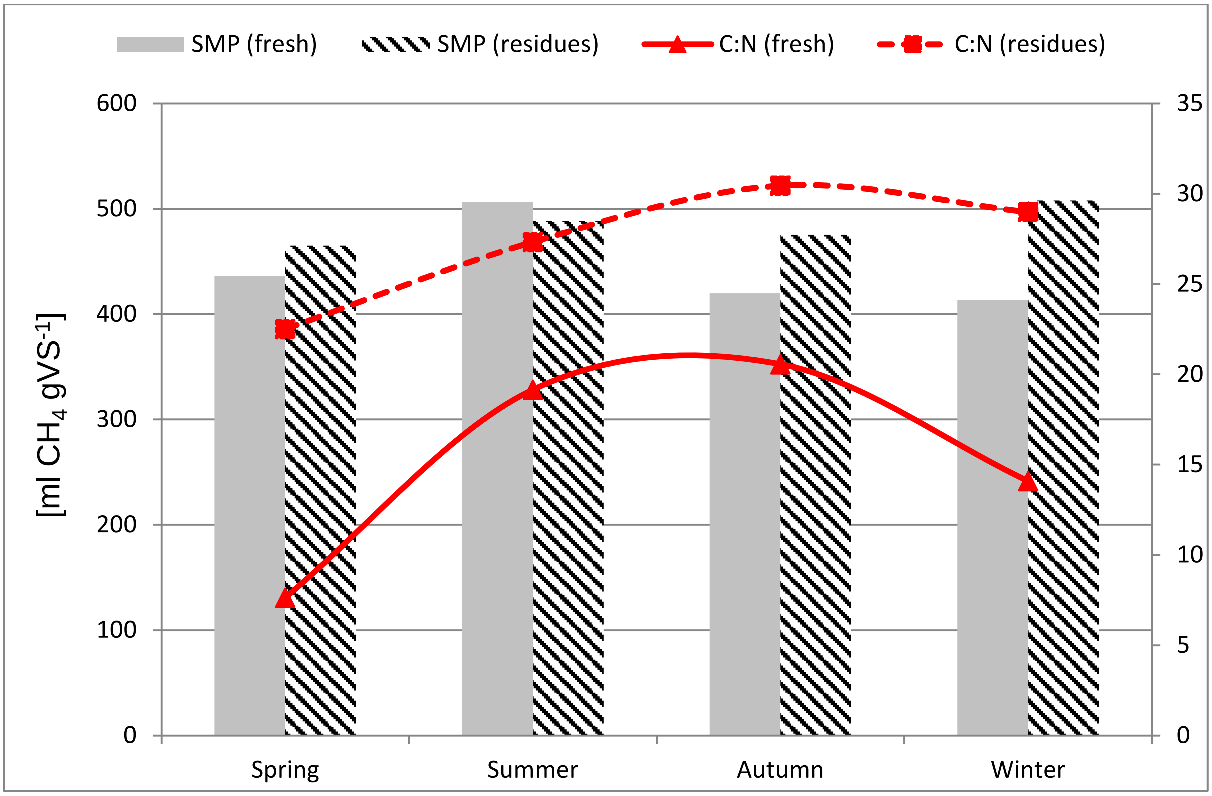


*Figure 1 Stoichiometric methane potential (SMP) of fresh and extracted LH samples*

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

## 3.2 BMP assays and effect of inoculum acclimatation

From the two-way ANOVA in Table 3, it can be observed that the model is significant and the following conclusions can be extrapolated from the analysis: (i) the means for S and AS are different, (ii) the means for each season are different and (iii) the interaction season/inoculum type has a very significant effect on methane production. Also, this is particularly confirmed for the AB-interaction as p-value is very small (0.0002). Results indicate that the summer season has a very high impact on BMP from S inoculum, while spring, summer and autumn are more determinant when using AS. Fit statistics indicate the Predicted $R^2$ of 0.6846 is in reasonable agreement with the Adjusted $R^2$ of 0.8521 as their difference is less than 0.2 and the model significance is confirmed by the value of adequate precision (>4) [25].

*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 | *significant* |
| 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.

Figure 2 (a) shows the model follows normal distribution of predicted versus actual values of methane yield in ml $CH_4$ $gVS^{-1}$ from the BMP assay, while Figure 2 (b) illustrates the interaction Season/Inoculum type (b) detected in the model by the 2-way ANOVA analysis. From the interaction plot, it can be noted summer's composition has the most significant impact on methane production, with best results achieved with acclimatation of inoculum (AS). Spring and autumn present a similar trend of methane production with slightly better yields (<100 ml $CH_4$ $gVS^{-1}$ difference) obtained in the autumn months using non-acclimatised inoculum (S). In winter the best yields are achieved with acclimatised inoculum with about 350 ml $CH_4$ $gVS^{-1}$.

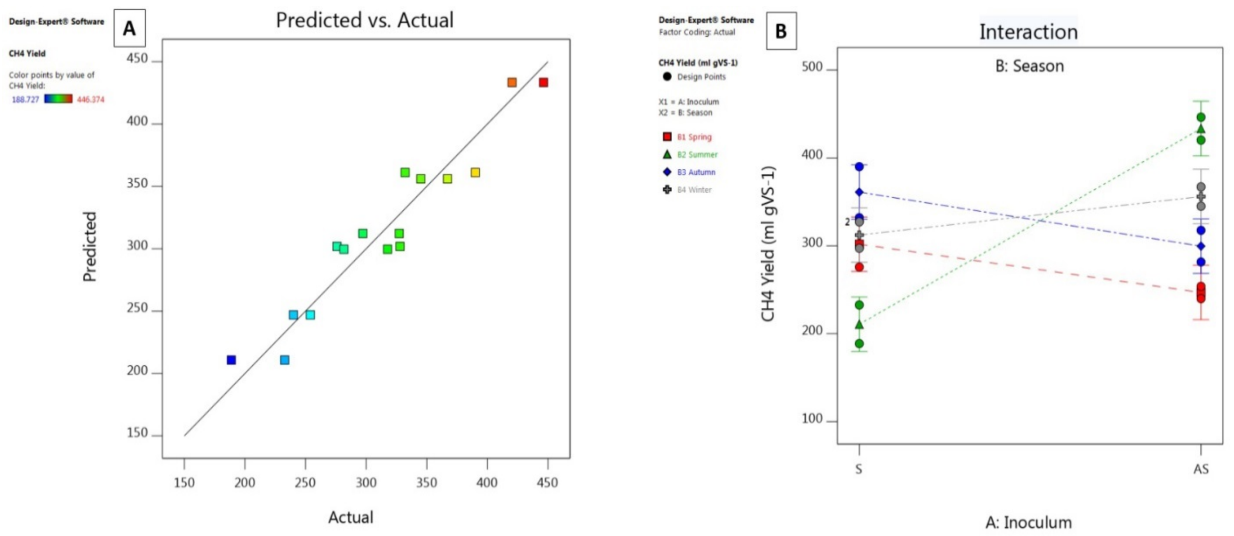


*Figure 2 Predicted vs Actuals normal distribution (a) and Interaction plot (b) from the two-way ANOVA*

Table 4 and Figure 3 summarise the results obtained by the BMP assays in terms of methane yields and overall biodegradation of the LH substrates in relation to the theoretical achievable yields (BMP/SMP). The best methane production occurs in the summer season using AS with a value of about 433 ml $CH_4$ $gVS^{-1}$ and very high final biodegradation (0.9). This yield is about 24.5% higher than volumes achieved from fresh *L. digitata* [16], which is currently considered the most promising for AD among brown species. Overall, by looking at the $\%CH_4$ in the biogas from the SMP and the actual BMP values, it can be observed the results from the BMP assay confirm such digestion yields are far better than mono-digestion [31]. These are higher on average +11.3% when using S and +9.5% when using AS when compared to the stoichiometric methane achievable. Highest average bioconversion rates in BI are instead obtained when using acclimatised (0.69) over non-acclimatised (0.61) inoculum, with a lead of +8% corresponding to 334 ml $CH_4$ $gVS^{-1}$. This result is higher than values obtained by [32] on fresh LH digestion however, the BMP was conducted in winter/spring, whose values are closer to those identified in this study. Results from Table 4 are in line with findings of [12, 33, 34]. Benefits of inoculum acclimatation at stabilising biomethane production rates from seaweed digestion have also been found by [35, 36].

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

| | | | S | | | AS | | |
|---|---|---|---|---|---|---|---|---|
| **Month of harvest** | **SMP [ml $CH_4$ $gVS^{-1}$]** | **CH4%** | **BMP [ml $CH_4$ $gVS^{-1}$]** | **Cumulative CH4%** | **BI (BMP/SMP)** | **BMP [ml $CH_4$ $gVS^{-1}$]** | **Cumulative CH4%** | **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 |

The lowest bioconversion rates can be identified in the summer when using S (0.43) and in the spring when using AS (0.53). In the first case, this result appears related to the A:V ratio which is the highest (0.42) reached by the substrate across the year, see Figure 4 (a), while acclimatation allows to overcome this obstacle for improved as well as maximised methane production. A similar behaviour of methane yielded in relation to A:V variations was observed by [14] on brown seaweed *Ascophyllum nodosum*. In addition, the sample is also characterised by relatively low tCOD, Figure 4 (b), which translates into less organics freely available to be hydrolysed by sugar-reducing bacteria. The latter reason is also behind the lowest methane yield obtained when using AS in spring, as tCOD is at its lowest. This affects significantly LH's bioconversion despite A:V being at its minimum. The adoption of a pretreatment in these particular instances is expected to improve tCOD concentrations, even by far [37-39], and therefore would be recommendable.

The highest methane production rates were expected in autumn due to the highest values of tCOD combined with a very low A:V ratio in the substrate. Resulting methane yields in this period were still high (in the rage of 299.6-361.1 ml $CH_4$ $gVS^{-1}$) with BI as high as 0.76. However, a yield lower than expectable can be justified by an overload in tCOD (53 g $ml^{-1}$) which is believed to have caused an inhibition of the methanogenesis phase. It is worth reporting that salt accumulation in this specific period has been found to inhibit methane production from digestion of fresh seaweed [14]. However, this should not be the case for the residues due to low A:V ratio detected (see Figure 4(a)).

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

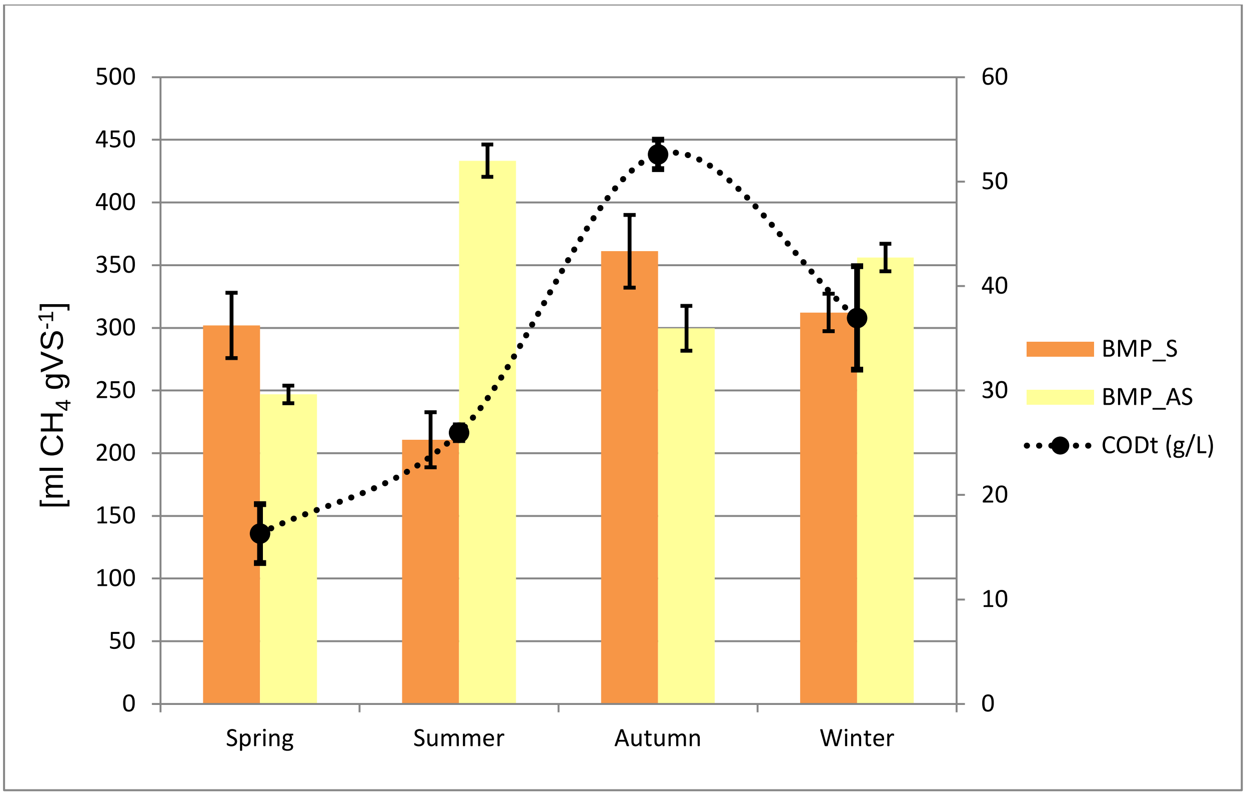


*Figure 3 BMP assay of L. hyperborea with and without inoculum acclimatation*

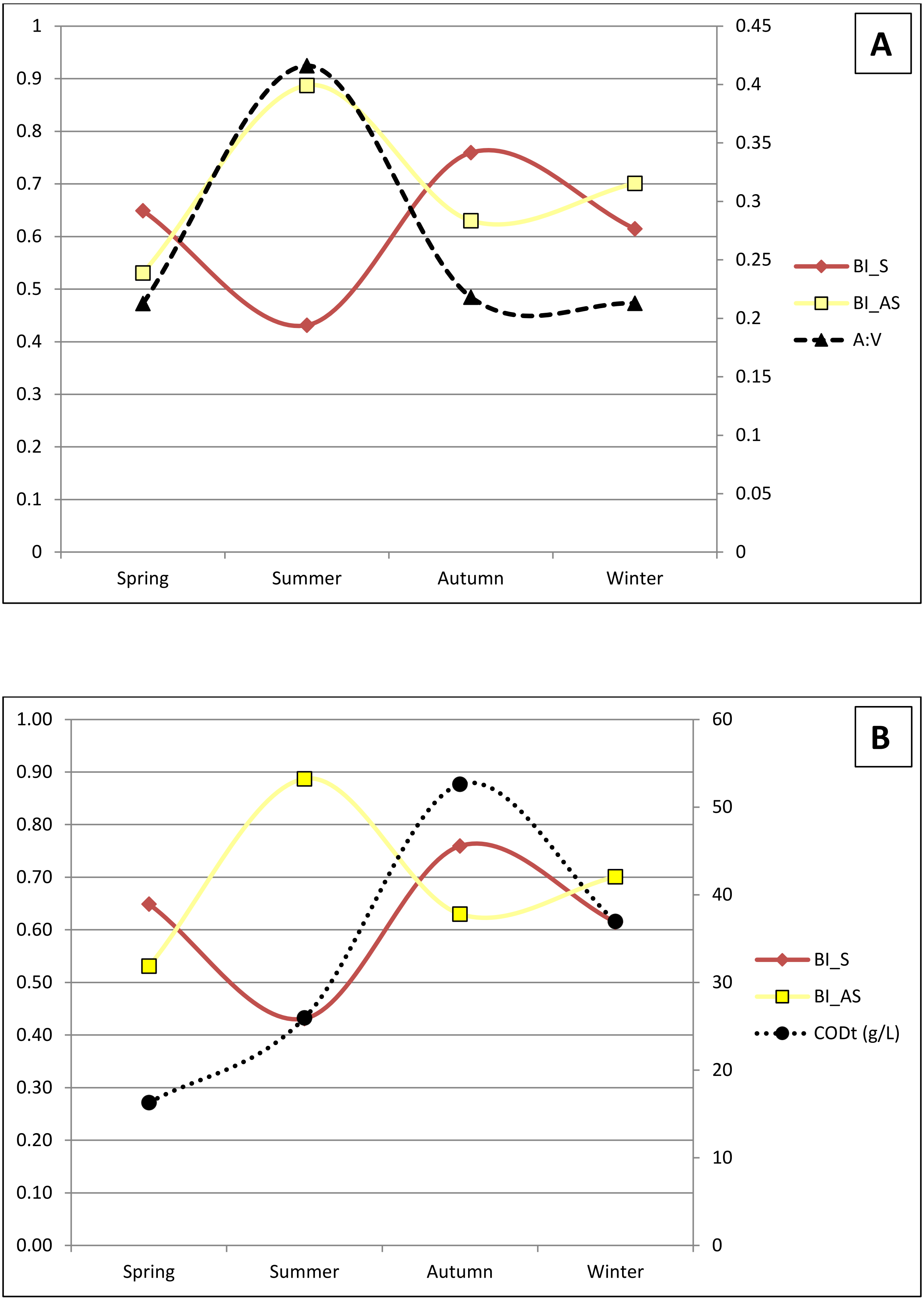


*Figure 4 Seasonality of BI indices from L. hyperborea's biodegradation against (a) A:V ratio and (b) tCOD concentration*

## 4. Conclusion:

This research aimed to determine the effect of seasonal variation in composition and inoculum acclimatation for the anaerobic digestion of brown seaweed *L. hyperborea* residues after ambient extraction cascade of a variety of bioproducts. The best methane production occurs in the summer season using acclimatised sludge with a value of about 433 ml $CH_4$ $gVS^{-1}$ and a bioconversion rate of 0.9. Methane yields from the BMP assays are higher on average +11.3% when using non-acclimatised sludge and +9.5% when using acclimatised if compared to the stoichiometric methane that can be achieved from the substrates.

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

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