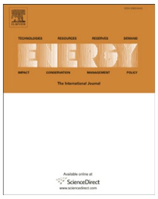
Energy 118 (2017) 1079–1089

Contents lists available at ScienceDirect

Energy

journal homepage: www.elsevier.com/locate/energy



Influence of mechanical pretreatment and organic concentration of Irish brown seaweed for methane production

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ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form
26 October 2016
Accepted 30 October 2016
Available online 14 November 2016

Keywords:
Laminaria sp.
Ascophyllum nodosum
Anaerobic digestion
Pretreatment
Organic substrate concentration

ABSTRACT

The European Commission opened a discussion about limiting first generation food based biofuels in favour of advanced biofuels. The main reason was to limit the uncertainty in estimates of indirect land use change emissions (ILUC) of food based biofuels. Brown seaweeds represent a valuable solution. The lack of lignin makes them suitable for degradation processes such as anaerobic digestion (AD). The main output of AD is biogas which can be upgraded to biomethane and used as a transport fuel. The most common Irish brown seaweeds namely *Laminaria* sp. and *Ascophyllum nodosum* were subject to AD. The effects of beating pretreatment time (5–10–15 min) and changes in the seaweeds volatile solids (VS) concentration (1–2.5–4%) on methane production were investigated through a response surface methodology (RSM). *Laminaria* sp. showed the highest methane yield of 240 ml CH_4 g^{-1} VS when the pretreatment time was set at 15 min and at VS concentration of 2.5%. In the case of *Ascophyllum nodosum*, the best yield of 169 mL CH_4 g^{-1} VS was found at the longest pretreatment time tested and at the minimum concentration of VS. The RSM analysis revealed that the VS concentration had the strongest impact on the methane yield.

1. Introduction

By 2020, Ireland has to achieve the target of 16% renewable energy of its total energy consumption as established by the EU Directive 2009/28/EC [1]. The National Renewable Energy Action Plan set out to fulfil the 16% overall target through 10% renewable energy supply in transport, 12% in the heat sector, and 40% in the electricity sector. Between 1990 and 2013 the contribution of renewable energy to the overall energy demand rose from 2.3% to 7.8% towards the 16% target, while 4.9%, 5.7% and 20.9% were reached in the transport, heat and electricity sector respectively [2]. According to a total projected demand from road and rail transport of 4.499 thousand of tonnes of oil equivalent (ktoe), it has been suggested a contribution of 33 ktoe from electric vehicles and of 406 ktoe from biofuels, in order to meet the 10% target in the transport sector by 2020 [3]. It is noteworthy that if the sources of biofuels are wastes, residues, non-food cellulosic material, lignocellulosic material or algae, the double weighting in the transport sector share can be applied [4,5]. The main reason is to limit the broad uncertainty in estimates of indirect land-use change (ILUC) impacts. These impacts occur when grassland and forest are converted to crop land somewhere on the globe to meet the demand for commodities displaced by the production of biofuel feedstocks. Thus, the climate benefits estimated for some biofuels can be negated [6]. The tendency is to call for biofuels derived from feedstocks such as wastes, residues, lignocellulosic biomass and certain algal production systems that do not involve displacing production of other commodities. The use of macroalgae, commonly known as seaweeds, for bioenergy conversion processes offers several advantages. This kind of biomass ensures high growth yields without requiring arable land [7–9], high capacity of carbon capture during photosynthesis [10] and a negligible or low amount of lignin makes them less resistant to degradation than lignocellulosic feedstock [11]. Thus, they are more suitable for degradation processes such as anaerobic digestion (AD) than land plants.

In particular, the life cycle assessment of algal biofuels suggests them to be environmentally better than the fossil fuels but economically it is not yet so attractive [12]. On this matter, Ghadiryanfar et al. [13] analysed the economics and the main

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http://dx.doi.org/10.1016/j.energy.2016.10.132
0360-5442/

advantages and drawbacks related to the use of seaweeds for biofuels along the entire supply chain.

The biogas produced through AD generally contains around 60% of methane which can be upgraded to biomethane. Biomethane can then be introduced into the gas grid or used as a transport fuel. Biogas is also used to power Combined Heat and Power (CHP) systems for heat and electricity production. Recent studies [14,15] showed that the use of biomethane as fuel can represent a valuable option for Ireland in order to meet the 10% target in transport sector through the use of indigenous energy sources. Ireland with over 7500 miles of coastlines and direct access to the Atlantic Ocean offers an ideal location to utilise seaweeds as a source of biofuel [16]. In particular, brown seaweeds such as *Laminaria* sp. and *Ascophyllum nodosum* are the most commercially important Irish seaweed species. About 16,000 tonnes of *Ascophyllum nodosum* are harvested each year in Ireland, dried and milled in factories at Arramara Teoranta, Cill Chiaráin (Kilkerrin), Co. Galway; and some 3000 t of the resulting seaweed meal is exported and processed in Scotland for the production of alginic acid. *Laminaria hyperborea* stipes are collected in drift in Scotland and Ireland and the rods are used for the manufacture of high-grade alginates [17].

The use of seaweeds for biogas production through AD has been technically evaluated by several works in the literature [18–20]. In the case of *Ascophyllum nodosum*, MacArtain et al. [21] registered a methane production around 176 ml g^{-1}VS, while Hanssen et al. [22] reported a biogas yield up to 280 ml g^{-1}VS with 50% of methane. *Laminaria* sp. methane yields were found ranging between 200 and 400 ml CH_4 g^{-1}VS [23–25]. These yields can be further improved by using a pretreatment step prior to AD. A series of different pretreatment technologies have been suggested [19]. The use of a mechanical pretreatment can be a viable route for seaweeds. The main effect of such pretreatment is to increase the substrate specific surface area and thus an increased access for degrading enzymes [26]. The result is to accelerate the start of the digestion, even though resulting in a marginal improvement of the overall methane production [26,27]. The main drawback is the high energy demand that in the case of seaweeds it is believed to be lower due to the lack of lignin [28]. Amongst mechanical pretreatments, the beating for seaweed biomass is the least studied. Tedesco et al. [29] showed that beating pretreatment applied to *Laminaria* sp. enabled to improve the biogas and methane yield of 52% and 53% respectively. Recently, it was showed that the beating pretreatment exhibited the highest methane yields when compared with other physical pretreatments such as microwave and ball milling [30]. On the other hand, the literature lacks of studies which investigated the effect of a mechanical pretreatment on the methane yield of *Ascophyllum nodosum*.

Several parameters influence the AD, one of the most important is the substrate concentration. It is known that an excessive substrate concentration leads to imbalances in the bacterial population, leading to VS accumulation and digester failure [31]. On the other hand, excessively low substrate concentration can result in starving conditions within the digester and a consequent reduced methane generation [32]. Only few studies have addressed the influence of substrate concentration on the AD of seaweeds so far [16,22,33]. In general, suitable substrate concentration must be investigated according to the nature and composition of algal substrate [19].

This study evaluated the influence of beating pretreatment and substrate concentration on AD of two common Irish seaweeds namely *Ascophyllum nodosum* and *Laminaria* sp. The pretreatment phase was tested in terms of beating time, while the substrate concentration was considered in terms of VS concentration. The response surface methodology (RSM) was used in order to evaluate the influence of beating time and VS concentration on methane production and the interaction between them. This technique allowed evaluating the possible interaction of influencing parameters on AD by limiting the number of planned experiments.

2. Materials and methods

2.1. Feedstocks and inoculum

A mixture of *Laminaria* sp. (*Laminaria digitata*, *Saccharina latissima*, and *Laminaria hyperborea*) was manually collected on shore in Howth (Dublin, Ireland), in early May 2014. From the same site *Ascophyllum nodosum* was manually collected in August 2014. Table 1 reports TS and VS contents for each species. Before pretreatment, fresh seaweeds were roughly cut and immediately treated without washing.

Digested sewage sludge was used as a source of inoculum. The sludge was collected from the Ringsend wastewater treatment plant (Celtic Anglian Water Ltd.), Dublin, Ireland operating at mesophilic temperature. The inoculum analysis revealed total solids (TS) content of 3.6 ± 0.5% Wt on its wet basis and a volatile solids (VS) concentration of 79.5 ± 4% Wt on its dry basis. The total Chemical Oxygen Demand (tCOD) and soluble COD (sCOD) were found equal to 60.15 ± 6.8 g O_2 L^{-1} and 5.8 ± 0.4 g O_2 L^{-1} respectively.

2.2. Mechanical pretreatment

Beating was performed as mechanical pretreatment by using a Hollander beater, model Reina. This kind of machine was originally built for the pulp and paper industry. It was equipped with a crank handle which allowed adjustment of the gap between the drum's blades and the bed-plate. The minimum gap achievable was 76 μm, which corresponded to one single turn of the crank handle. In general, the machine performs two main actions; (a) – cutting action caused by the grooves located on the bed-plate, and (b) – high pressure beating action of the feedstock against an inclined plate placed at the exit-out of the drum. The drum of the machine permitted a constant rotational speed of 580 rpm. Even though, the machine was capable to operate both wet and dry biomass, it was necessary to add water in order to cause the recirculation of the feedstock. The result was a pulp of different consistencies according to the gap and the processing time applied. In this experimental work, the machine was operated at the minimum gap of 76 μm for each beating time (5, 10, 15 min) under investigation.

2.3. TS and VS analysis

The TS amount was determined by drying the samples at 105 °C until constant weight, while the VS fraction was assessed through combustion of a known weight dried sample at 575 ± 25 °C until constant weight, according to standard methods (NREL/MRI LAP 1994, 2008).

2.4. Total and soluble COD

Total (tCOD) and soluble (sCOD) COD were determined through the colorimetric method. For COD analysis the procedure followed

Table 1
TS and VS analysis.

Species	TS [% Wt on wet basis]	VS [% Wt of TS]
Laminaria sp.	14 ± 1	66 ± 8
Ascophyllum nodosum	30 ± 3	73 ± 5

is reported as Hach method 8000 for water, wastewater and seawater by Hach Lange Company. The measurements were carried out using Hach standard kit (range 0–1500 mg/L, Hach Lange, Düsseldorf, Germany) and a Hach Lange DR2000 spectrometer to read the samples. Prior to sCOD determination, a vacuum filtration through a glass microfiber filter (1.5 μm of pore size) at first and then through a membrane filter (0.1 μm of pore size) was performed. Both tCOD and sCOD were determined by diluting the samples at a dilution factor of 1:100.

2.5. Anaerobic biodegradability

A batch system was used as the AD experiment set-up. The bioreactors consisted of borosilicate glass flasks of 500 ml in capacity. Each bioreactor was filled with 200 ml of treated seaweed at different VS contents such as 1, 2.5 and 4%, and 200 ml of sludge at a constant VS content of 3%. The total liquid volume was 400 ml. Samples of untreated seaweed for each different VS concentration were also included. Samples of sludge-only were digested and the amount of biogas produced was then subtracted from the co-digesting yields. All samples were carried out in duplicate. The reactors were then sealed with borosilicate glass adapters equipped with controlled gas opening valves and purged with nitrogen flow for 5 min in order to achieve anaerobic conditions. The incubation time was set at 14 days. The biogas produced during the reaction was collected in airtight Linde plasti-gas bags and collected after 6 days and at the end of digestion. At each collection the biogas volume was then measured by using gas sampling tubes which were installed in a gas jar with confining liquid according to procedure VDI 4630 [34]. Before and after incubation, the pH for each sample was measured by using a Hanna precision pH meter (accuracy ± 0.01), model pH 213. Waterbaths were used to incubate the reactors at an operating mesophilic temperature of 38 ± 1 °C. During incubation, the bioreactors were shaken manually once a day. A biogas analyser, model Drager X-am 7000, was used to verify that the system was anaerobically isolated, and to measure the percentage of CH_4 in the biogas. The entire experiment set-up is represented in Fig. 1.

2.6. Response surface methodology (RSM)

The RSM used in the present study was a face-centred central composite design (FCCD) involving two numeric factors (A: time of pretreatment and B: VS concentration) and a categorical factor (C: seaweed species). The levels values for each variable are reported in Table 2. These were selected by considering previous studies on the subject. Tedesco et al. [29] investigated a beating pretreatment on *Laminaria* sp. by testing a range between 5 and 15 min as time of pretreatment, in this case the best result in terms of methane production was observed after 10 min of pretreatment, while no studies are available on the use of a beating pretreatment for *Ascophyllum nodosum*. Regarding the organic matter concentration, Hanssen et al. [22] found out that the optimum methane production from *Laminaria* sp. and *Ascophyllum nodosum* was achieved with a VS concentration below 6%. According to these results, a centre point at 10 min and 2.5% of VS concentration was designed. Therefore, for each seaweed species, a total of 13 experiments were conducted with the first 9 experiments organized in a 3^2 full factorial design with two operating variables and the remaining 4 involving the replications of the centre point. A total of 26 runs were performed.

The ANOVA was used in order to check the adequacy of the model developed and to obtain the interaction between the process variables and the response. The quality of the polynomial model fit was expressed by the coefficient of determination R^2, and its statistical significance was checked by the Fisher's *F*-test. Model terms were evaluated by the *p*-value with 95% confidence level ($\alpha = 0.05$). The statistical analysis was carried out by using the Design-Expert software (version 9.0.3.1).

2.7. Optimisation: pretreatment's energy evaluation

An important tool offered by Design-Expert software was the possibility to optimise the response while this was subject to specific constraints of the independent variables. This approach is known as a constrained optimisation problem [35]. Two optimisation problems were considered:

- maximising the methane yields.
- maximising the methane yields while minimising the time of pretreatment.

A comparison between the two solutions generated by the software was carried by considering the electricity consumption of the beating machine during the experiment. Thus, the following formulas were employed:

$$B_S = CH_4\ [\%] * \frac{9.67}{97} \tag{1}$$

In the above equation, B_s [kWh m^{-3}] is the energy content of the biogas produced by seaweed, CH_4 [%] is the average percentage content of methane of biogas produced by seaweed, 9.67 kWh is the energy content of 1 m^3 of biogas at 97% content of methane [36].

$$E_p = B_p * B_s \tag{2}$$

In the above equation, E_p [Wh g^{-1}VS] is the energy related to the biogas produced from 1 g of VS of seaweed and B_p [m^3 g^{-1}VS] is the quantity of biogas produced from each gram of VS of seaweed.

$$E_C = \frac{E_{pt}}{VS_m} \tag{3}$$

In the above equation, E_C [Wh g^{-1}VS] is the energy consumed by the pretreatment in order to process 1 g of VS of seaweed, E_{pt} [Wh] is the energy consumed during the pretreatment measured by a kilowatt hour meter, VS_m [g] is the total amount of VS into the machine.

$$Net\ E_P = E_P - E_C \tag{4}$$

The $Net\ E_P$ [Wh g^{-1}VS] is the energy produced by 1 g of VS of seaweed treated by taking into account the energy consumed by the pretreatment.

3. Results and discussion

3.1. Methane production

Figs. 2–3 and Table 3 report the cumulative methane yields registered for both species at different experimental combination after 14 days of digestion. The experimental error was reported as standard deviation calculated between measurements. A graphical appreciation of such error is reported as bars in Figs. 2–3. *Laminaria* sp. yielded higher methane than *Ascophyllum nodosum* for all experimental combinations. In terms of methane content, most of *Laminaria* sp. samples exhibited an average of 50% of CH_4, with a peak of 70% for the untreated and a minimum of 20% for the highest VS concentration of 4%. On the other hand, *Ascophyllum nodosum* exhibited a constant average of 40–45% of CH_4 along all the samples. It was observed that the behaviour of the treated samples with

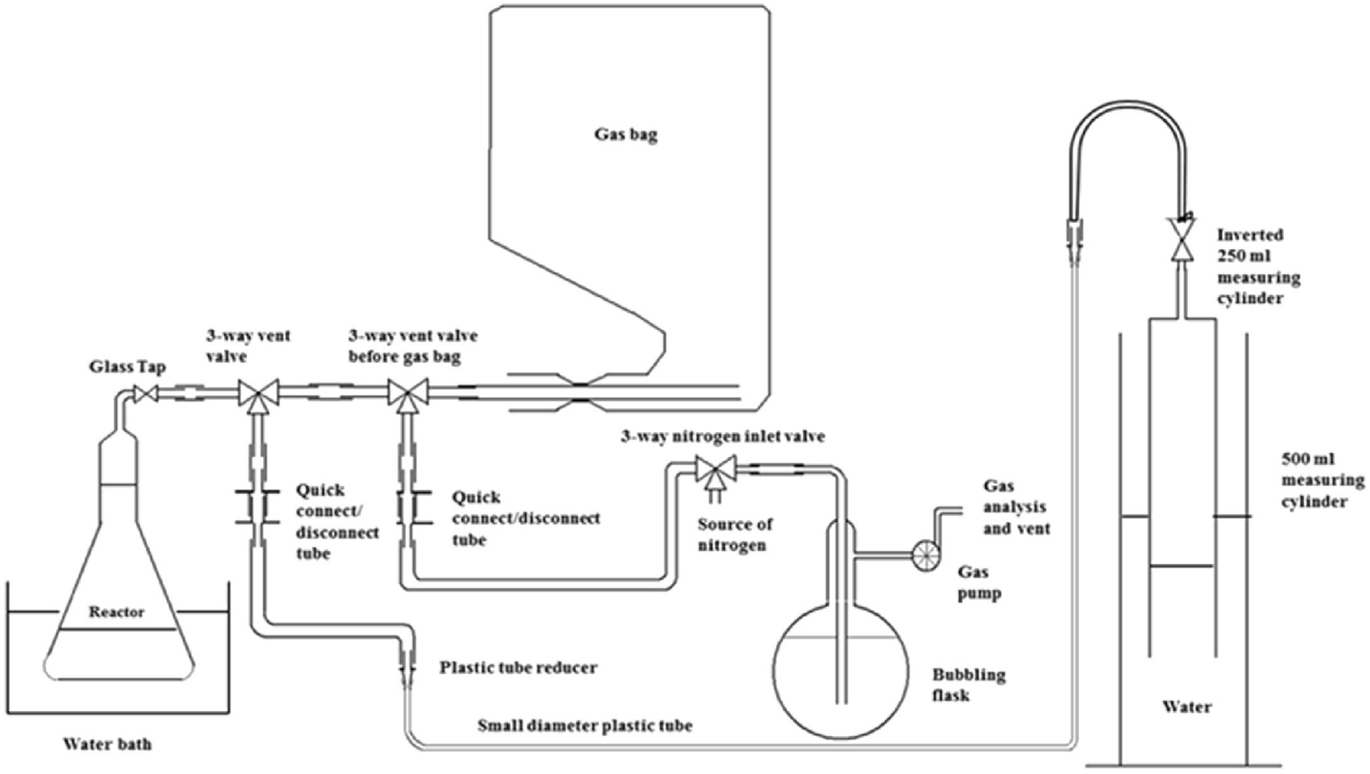

Fig. 1. AD experiment set-up (2-column).

respect to the untreated condition depended mainly on the algal species. *Ascophyllum nodosum* treated for 15 min and at 1% VS yielded up to 30% more methane than the untreated sample, while *Laminaria* sp. showed about 9% more methane than the untreated samples only at 2.5% of VS and after 10 and 15 min of beating.

Both species exhibited the lowest methane yields at the highest level of VS concentration.

At 4% VS, the sCOD values measured for *Laminaria* sp. (Table 3), indicated that not all the available organic matter went through the digestion process. The final sCOD (around 5 g O L^{-1}) was higher with respect to other experimental conditions which exhibited a final sCOD in the range of 2–3 g O L^{-1}. This suggests that the use of longer retention time can be beneficial in order to allow a more complete consumption of the degradable substrate.

On the contrary, *Ascophyllum nodosum* at 4% VS exhibited a final sCOD in the same range of the other samples which yielded higher methane. In this case, it is likely that an inhibition occurred as consequence of an overloading of the digester as most of the degradable organic matter was transformed into other co-products than methane. The consequence was a failure in methane

Table 2
Variables matrix.

Variable under investigation-factor	Levels	Response
Seaweed species (categorical)	1. *Laminaria* sp. 2. *Ascophyllum nodosum*	Methane production [ml g^{-1} VS]
VS concentration (numeric)	1. 1% 2. 2.5% 3. 4%	
Beating time (numeric)	1. 5 min 2. 10 min 3. 15 min	

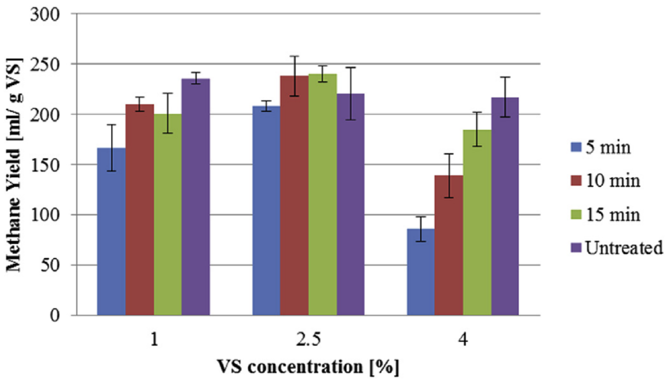

Fig. 2. *Laminaria* sp. methane yields (single column).

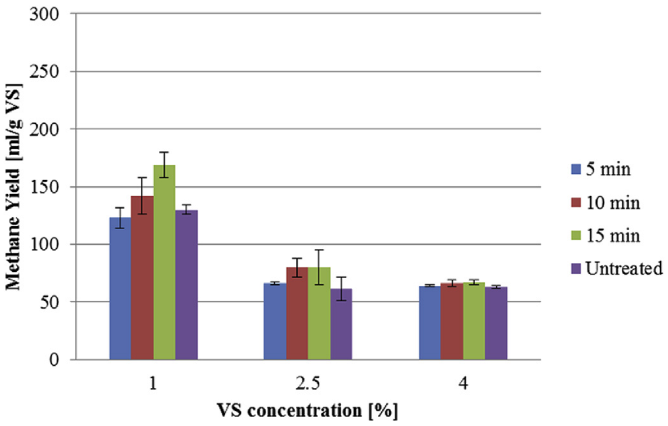

Fig. 3. *Ascophyllum nodosum* methane yields (single column).

Table 3
Methane yields, biogas yields, sCOD for *Laminaria* sp. and *Ascophyllum nodosum*.

Sample		*Laminaria* sp.				*Ascophyllum nodosum*			
VS [%]	BT [min]	Initial sCOD [g O L^{-1}]	Final sCOD [g O L^{-1}]	CH_4 [ml g^{-1} VS]	Biogas [ml g^{-1} VS]	Initial sCOD [g O L^{-1}]	Final sCOD [g O L^{-1}]	CH_4 [ml g^{-1} VS]	Biogas [ml g^{-1} VS]
1	0	N.A.	N.A.	236 ± 6	482 ± 8	N.A.	N.A.	130 ± 4	315 ± 8
1	5	5.08 ± 0.48	2.7 ± 0.33	167 ± 23	402 ± 20	4.67 ± 0.16	3.19 ± 0.11	123 ± 9	294 ± 23
1	10	4.78 ± 0.28	2.08 ± 0.38	210 ± 7	491 ± 10	4.88 ± 0.21	3.41 ± 0.31	142 ± 16	337 ± 28
1	15	5.03 ± 0.36	2.68 ± 0.27	201 ± 20	463 ± 25	4.40 ± 0.19	3.39 ± 0.26	169 ± 11	402 ± 20
2.5	0	N.A.	N.A.	221 ± 26	451 ± 24	N.A.	N.A.	61 ± 10	150 ± 29
2.5	5	5.63 ± 0.61	2.80 ± 0.46	208 ± 5	433 ± 1	6.07 ± 0.27	3.33 ± 0.11	73 ± 1	177 ± 4
2.5	10	6.30 ± 0.21	2.93 ± 0.29	238 ± 20	494 ± 22	6.62 ± 0.13	3.61 ± 0.33	80 ± 8	193 ± 19
2.5	15	5.53 ± 0.96	2.2 ± 0.55	240 ± 8	615 ± 7	6.55 ± 0.12	3.26 ± 0.38	80 ± 15	189 ± 29
4	0	N.A.	N.A.	217 ± 20	413 ± 18	N.A.	N.A.	63 ± 1	156 ± 2
4	5	7.60 ± 0.39	5.8 ± 0.26	86 ± 12	222 ± 23	7.83 ± 0.05	3.43 ± 0.22	64 ± 1	156 ± 3
4	10	7.53 ± 1.13	4.63 ± 0.49	139 ± 22	317 ± 26	9.02 ± 0.73	3.45 ± 0.25	66 ± 3	161 ± 8
4	15	7.08 ± 0.79	4.58 ± 0.68	185 ± 17	374 ± 25	10.75 ± 0.52	4.18 ± 0.45	67 ± 2	164 ± 6

production, with a reduction of the sCOD as most of the organic matter was used for the microbial activity. Methane yields after 6 days of digestion are reported in Table 5. The *Laminaria* sp. data revealed that at 4% of VS, a hampering of the digestion was caused by the pretreatment since much higher yields were observed for the untreated samples. Besides, for all the treated samples at 4% an initial pH above 7 was measured (Table 4), whilst for the untreated sample the pH resulted equal to 7.47 ± 0.01 which was more suitable for AD. Thus, it was likely that the enhanced solubilisation of the organic matter caused by the beating pretreatment determined a decrease in pH with respect to the untreated samples. However, since the treated samples after 14 days of digestion exhibited a suitable pH (ranging between 7.40 and 7.58) with high sCOD values, it was probable that the buffer capacity of the system was sufficient in order to allow the anaerobic microorganisms to survive and adapt. Thus, at 4% of VS longer retention times after pretreatment would allow for a better performance of the digester.

At 2.5% of VS, there was an increase of 50% methane for all the treated samples. Such increase of methane suggests that the main effect of the beating pretreatment was to accelerate the start of digestion while resulting in a marginal methane enhancement at the end of digestion.

Unlike *Laminaria* sp., at 6 days of digestion *Ascophyllum nodosum* did not exhibit an enhancement of methane after pretreatment even though a general improvement in the methane yields of treated samples with respect to the untreated was observed at the end of the incubation time.

According to these results, it is evident that the pretreatment phase impacted differently according to the seaweed species used as well as the VS concentration. The RSM analysis was carried out in order to evaluate the impact of the pretreatment and VS concentration on the methane response according to the seaweed species.

Table 4
pH values for *Laminaria* sp. and *Ascophyllum nodosum*.

Sample		*Laminaria* sp. pH		*Ascophyllum nodosum* pH	
VS [%]	BT [min]	Initial	Final	Initial	Final
1	0	7.44 ± 0.03	7.41 ± 0.02	7.92 ± 0.03	7.44 ± 0.02
1	5	7.27 ± 0.04	7.52 ± 0.03	7.96 ± 0.03	7.45 ± 0.04
1	10	7.25 ± 0.01	7.37 ± 0.02	7.93 ± 0.01	7.41 ± 0.01
1	15	7.28 ± 0.02	7.36 ± 0.04	7.99 ± 0.03	7.44 ± 0.02
2.5	0	7.45 ± 0.02	7.40 ± 0.02	7.90 ± 0.02	7.33 ± 0.01
2.5	5	7.07 ± 0.01	7.59 ± 0.01	7.71 ± 0.01	7.34 ± 0.05
2.5	10	7.07 ± 0.02	7.61 ± 0.04	7.81 ± 0.04	7.38 ± 0.06
2.5	15	7.04 ± 0.04	7.60 ± 0.05	7.84 ± 0.02	7.31 ± 0.01
4	0	7.47 ± 0.01	7.45 ± 0.01	7.82 ± 0.03	7.31 ± 0.01
4	5	7.03 ± 0.02	7.40 ± 0.03	7.48 ± 0.03	7.46 ± 0.01
4	10	6.98 ± 0.02	7.69 ± 0.03	7.46 ± 0.01	7.39 ± 0.02
4	15	6.93 ± 0.05	7.58 ± 0.01	7.47 ± 0.01	7.41 ± 0.04

3.2. Model estimation

The RSM design matrix with the methane response for each combination of factors levels is shown in Table 6.

The ANOVA table as yielded by the software (Table 7) showed that the estimated model was significant as well as the model terms A, B, C, BC, A^2, ABC and A^2C. At the same time, the *p*-value related to the “Lack of Fit”, was >0.05, which implied that the “Lack of Fit” was not significant. This meant that the model developed adequately fit the data.

The values of R^2, adjusted R^2 (*Adj.* R^2), and predicted-R^2 (*Pred.* R^2) were all close to 1, which indicated that the chosen model was adequate to predict the CH_4 yields from the variables within the experimental boundaries. An adequate precision (*Adeq. Precision*) greater than 4 indicated that this model could be used to navigate the design space.

Equation (5) represents the final model equation in terms of coded factor. By default, the software encoded the high levels of the factors as +1 and the low levels of the factors as −1 (Table 8). The equation was calculated by the software and obtained for the CH_4 yield (Y) as a function of the independent variables A (VS concentration), B (beating time) and C (species).

$$Y = +158.60 - 33.75\,A + 18.42\,B - 79.67\,C + 2.75\,AB - 5.75\,AC - 9.08\,BC - 18.61\,A^2 - 7.61\,B^2 - 3.5\,ABC + 45.35\,A^2C + 6.85\,B^2C \tag{5}$$

By comparing the factors' coefficients, the species selected (C) represented the highest impact on the response. When *Laminaria* sp. was selected, the impact of the relative coefficient on methane production resulted to be positive, while the impact was negative in the case of *Ascophyllum nodosum*. The other two strong impacts were the interaction A^2C and the VS concentration (A) respectively. In the case of the A^2C term, the impact was dependent on the value of the C term (negative for *Laminaria* sp. and positive for *Ascophyllum nodosum*), while the term A has a positive impact at low VS concentrations.

The software computed the final equations (Eqs. (6) and (7)) in terms of actual factors for *Laminaria* sp. and *Ascophyllum nodosum* respectively:

$$Y = +48.57 + 101.81\,A + 11.66\,B + 2.17\,AB - 28.43\,A^2 + 0.58\,B^2 \tag{6}$$

Table 5
Laminaria sp. and *Ascophyllum nodosum* methane yields at 6 days of digestion.

Sample		*Laminaria* sp. at 6 days of digestion		*Ascophyllum nodosum* at 6 days of digestion	
VS [%]	BT [min]	CH$_4$ [ml g^{-1} VS]	Treated vs Untreated [%]	CH$_4$ [ml g^{-1} VS]	Treated vs Untreated [%]
1	0	150 ± 2		93 ± 1	
1	5	128 ± 10	−15	73 ± 7	−22
1	10	128 ± 8	−15	78 ± 8	−16
1	15	116 ± 19	−23	96 ± 8	3
2.5	0	104 ± 14		52 ± 9	
2.5	5	159 ± 21	53	54 ± 3	4
2.5	10	160 ± 19	54	59 ± 5	13
2.5	15	161 ± 9	55	40 ± 20	−23
4	0	140 ± 10		57 ± 1	
4	5	23 ± 6	−509	50 ± 4	−12
4	10	33 ± 4	−324	51 ± 4	−11
4	15	55 ± 3	−155	53 ± 1	−7

Table 6
Design matrix with methane response.

Exp. No.	Factors			
	A: VS concentration [%]	B: beating time [min]	C: Seaweed species	Response (Y): Methane [ml g^{-1} VS]
1	1	15	*Laminaria*	201
2	2.5	10	*Ascophyllum nodosum*	66
3	2.5	10	*Laminaria*	270
4	4	15	*Ascophyllum nodosum*	67
5	2.5	10	*Laminaria*	216
6	2.5	10	*Ascophyllum nodosum*	86
7	4	10	*Ascophyllum nodosum*	66
8	4	15	*Laminaria*	185
9	2.5	10	*Ascophyllum nodosum*	74
10	1	15	*Ascophyllum nodosum*	169
11	2.5	15	*Laminaria*	240
12	4	5	*Laminaria*	86
13	2.5	10	*Laminaria*	248
14	2.5	10	*Laminaria*	237
15	2.5	10	*Ascophyllum nodosum*	89
16	2.5	10	*Ascophyllum nodosum*	83
17	2.5	5	*Laminaria*	208
18	4	10	*Laminaria*	139
19	1	10	*Ascophyllum nodosum*	142
20	1	5	*Ascophyllum nodosum*	123
21	1	5	*Laminaria*	167
22	2.5	5	*Ascophyllum nodosum*	73
23	2.5	15	*Ascophyllum nodosum*	80
24	4	5	*Ascophyllum nodosum*	64
25	2.5	10	*Laminaria*	220
26	1	10	*Laminaria*	210

Table 7
ANOVA table.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.194E+005	11	10853.43	50.98	<0.0001	significant
A: VS [%]	13668.75	1	13668.75	64.20	<0.0001	
B: BT [min]	4070.08	1	4070.08	19.12	0.0006	
C: Species	73633.24	1	73633.24	345.84	<0.0001	
AB	60.50	1	60.50	0.28	0.6024	
AC	396.75	1	396.75	1.86	0.1938	
BC	990.08	1	990.08	4.65	0.0489	
A^2	1913.50	1	1913.50	8.99	0.0096	
B^2	320.07	1	320.07	1.50	0.2404	
ABC	1458.00	1	1458.00	6.85	0.0203	
A^2C	11362.12	1	11362.12	53.37	<0.0001	
B^2C	259.45	1	259.45	1.22	0.2883	
Residual	2980.76	14	212.91			
Lack of Fit	690.76	6	115.13	0.40	0.8589	Not significant
Pure Error	2290.00	8	286.25			
Cor Total	1.224E+005	25				

$R^2 = 0.9756$; Adj. $R^2 = 0.9565$; Pred. $R^2 = 0.9157$; Adeq. Precision = 18.896.

Table 8
Variables coded factors.

Variable	Coded factors		
	−1	0	+1
A: VS concentration [%]	1	2.5	4
B: Beating Time [min]	5	10	15
C: Species	*Laminaria* sp.	N.A.	*Ascophyllum nodosum*

$$Y = +161.51 - 71.43\,A + 6.06\,B - 1.44\,AB + 11.89\,A^2 - 0.03\,B^2 \quad (7)$$

Fig. 4 shows the normal probability of residuals. Since the plotted dots resembled a straight line, it was assumed that the underlying error distribution was normal and therefore, the ANOVA procedure could be considered as an exact test of hypothesis of no difference in treatment means. A possible problem could be represented by the red point at the far right of the graph. It could be an outlier and therefore required further investigation. Thus, the Design-Expert diagnostics tool was run. Fig. 5 showed that the standard deviation of such point (highlighted point) was very low, indicating that this was not the case of an outlier.

In any case, the predicted values versus the actual values (Fig. 6) plot showed a good prediction of the model as most of the points were grouped around the diagonal line. This meant that there was a strong correlation between the model's predicted results and the actual results.

The resulting surfaces for each species and the correspondent contour plots are represented in Figs. 7–8. In the case of *Laminaria* sp. (Fig. 7). the optimum region for methane production was visible around the centre point (2.5%) of the VS concentration factor and in correspondence of the highest level of the beating time factor. Whilst for *Ascophyllum nodosum* (Fig. 8), the methane yield increased as the VS concentration reduced and the beating time increased.

An immediate investigation of such trends was possible through the perturbation plots (Figs. 9 and 10). The perturbation plots displayed the effect of changing each factor while holding the other one constant. The curvature of the VS concentration (*A*) factor for both species suggested that this factor influenced the methane yield response more than the time of pretreatment (*B*). The higher impact of the VS concentration relative to the beating time factor was also confirmed by the correspondent coefficients in the general model Equation (5). In particular, for both species the methane yield decreased when the VS concentration increased from the centre point (2.5%) up to the highest level (4%). *Laminaria* sp. exhibited the best methane yields when the VS concentration ranged between 1.75% and 2.5%, whilst for *Ascophyllum nodosum*, the methane yield increased dramatically from the centre point (2.5%) up to the minimum level (1%).

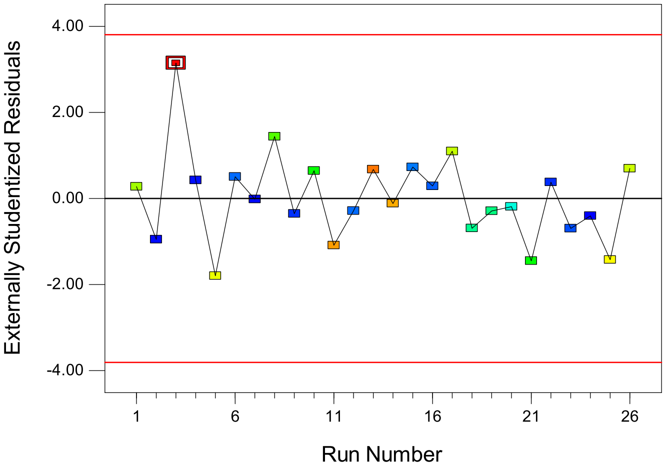

Fig. 5. Residual vs Run (single column).

Increasing the beating time (*B*) influenced positively the methane yield for both species. The impact of such factor was more important in the case of *Laminaria* sp. as a slight curvature was observable with respect to the *Ascophyllum nodosum* plot.

Figs. 11 and 12 represent the *AB* interaction plot for *Laminaria* sp. and *Ascophyllum nodosum*. It is interesting to notice that when the VS concentration was set at 4%, in the case of *Ascophyllum nodosum*, the beating time had almost no effect on the response, while for *Laminaria* sp. an increase of beating time determined an increase in the methane yield. At this concentration, the pretreatment phase seemed to have the strongest impact on *Laminaria* sp., even though resulting in lower methane yields compared to lower levels of VS.

For both species, at the lowest level of VS concentration (1%), the methane yields were higher compared to a 4% of VS. Unlike *Laminaria* sp., *Ascophyllum nodosum* interaction plot did not show any overlapping between the least significance difference (LSD) intervals at 5 and 15 min, thus the predictions at those points were significant. Therefore, at 1% of VS concentration it was possible to

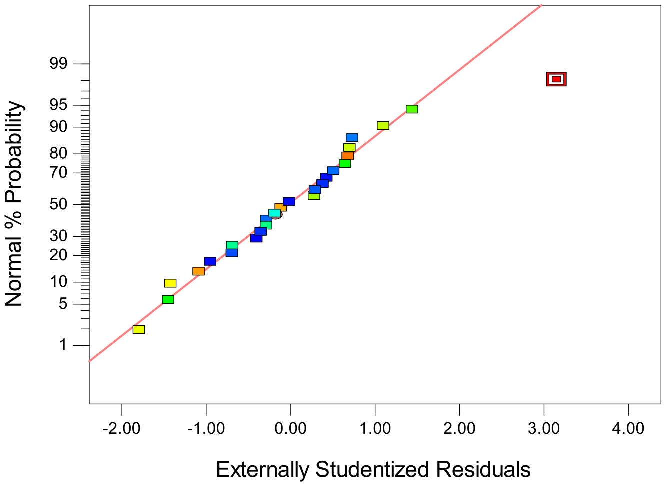

Fig. 4. Normal probability plot (single column).

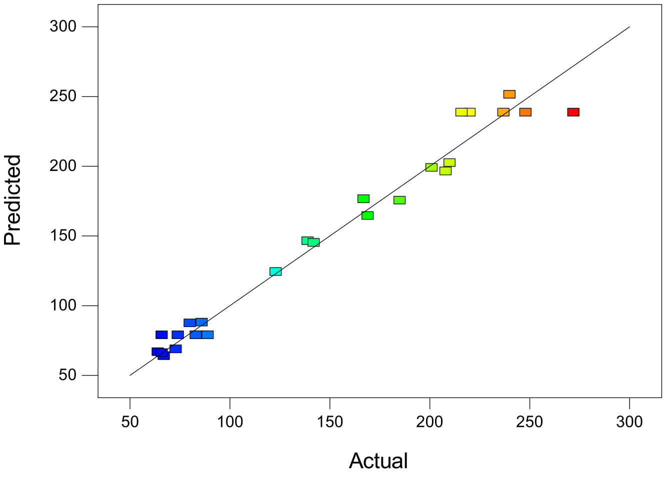

Fig. 6. Predicted vs Actual residuals (single column).

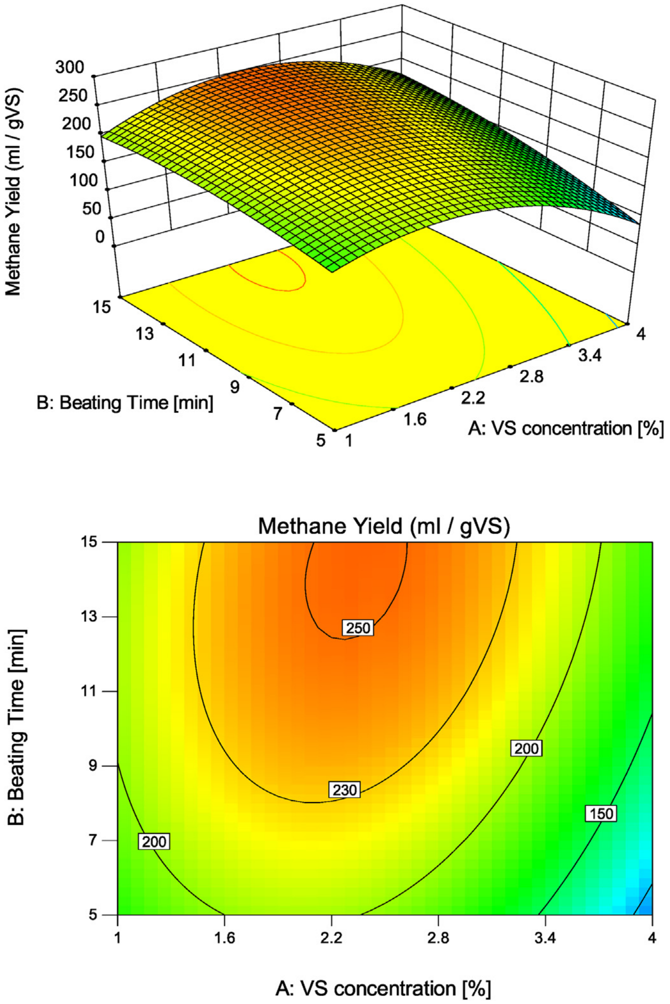

Fig. 7. *Laminaria* sp. response surface and contour plot (single column).

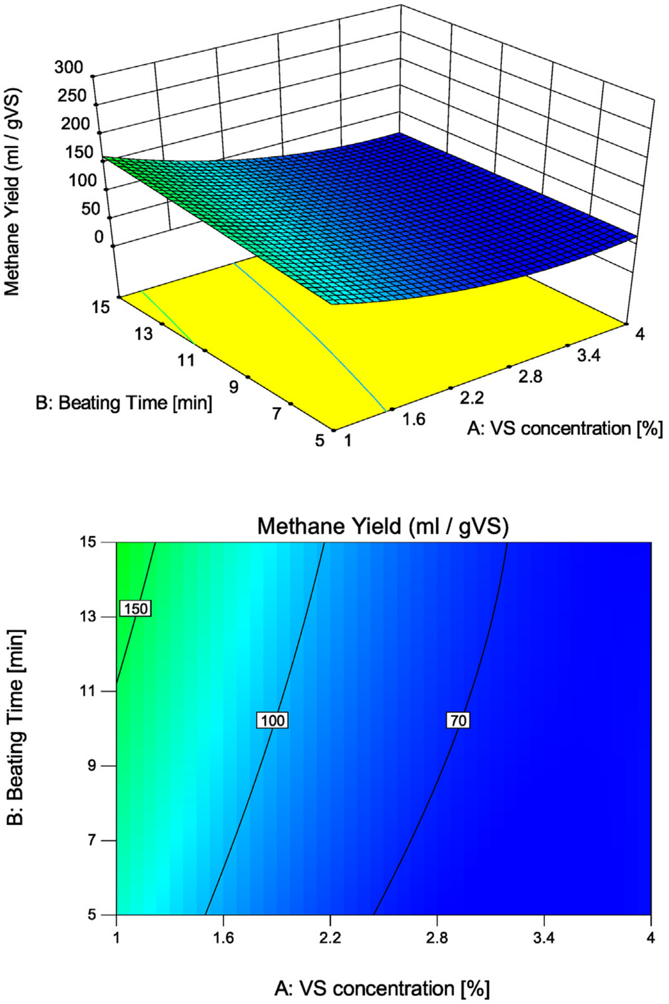

Fig. 8. *Ascophyllum nodosum* response surface and contour plot (single column).

improve the methane production from *Ascophyllum nodosum* by enhancing the time of beating up to 15 min. On the other hand, when treating *Laminaria* sp. at 1% of VS, there was no statistical evidence which suggested that an enhancement of pretreatment time improved significantly the methane yield from this species.

The pretreatment phase had the strongest impact on *Laminaria* sp. when the VS concentration was set at 4% even though resulting in lower methane yields compared to lower levels of VS. Nevertheless, it is interesting to note that, at 15 min of pretreatment there was no significant difference between the methane yields reached at 1% and 4% of VS. Thus, when increasing the beating time to 15 min, the influence of the VS concentration on the methane yields from *Laminaria* sp. did not have any effect.

Fig. 13 shows the *BC* interaction plot when the VS concentration was set at 2.5%. Both at 5 and 15 min there was no overlapping from left to right of the LSD bars, which means that between species there was a significant difference in methane yields at those two levels of treatment time. In the case of *Ascophyllum nodosum*, since there was an overlap between the LSD bars at 5 and 15 min, at 2.5% the pretreatment phase did not have any significant effect on the methane yield of this species, unlike *Laminaria* sp. which showed a significant difference between the yields at 5 and 15 min, with a better performance at 15 min.

The results showed that *Laminaria* sp. produced up to 240 ml g^{-1} VS, while *Ascophyllum nodosum* reached up to 169 ml g^{-1} VS, which corresponded to 40% more methane from *Laminaria* sp. The observed difference between the two species could be explained by the presence of polyphenols. Polyphenols are known for their inhibitory action towards microbial activities, mainly due to inhibition of vital enzymes [37]. Moen et al. [38] found out that a limiting factor for the conversion of organic

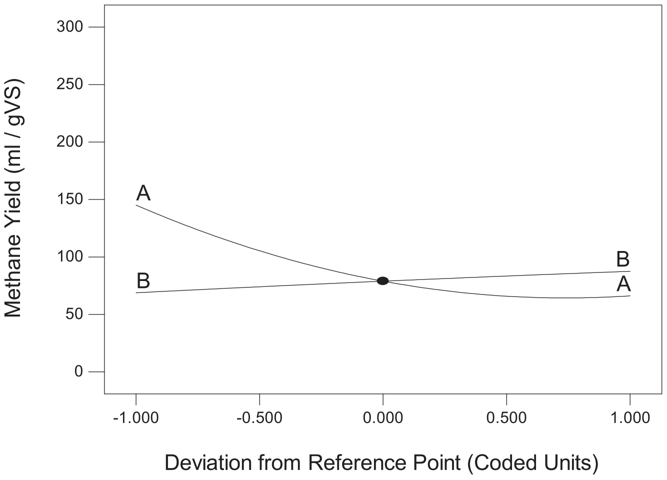

Fig. 9. Perturbation plot *Ascophyllum nodosum* (single column).

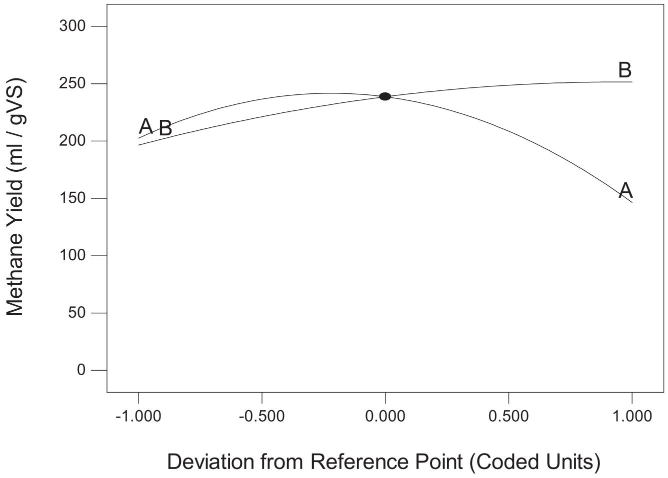

Fig. 10. Perturbation plot *Laminaria* sp. (single column).

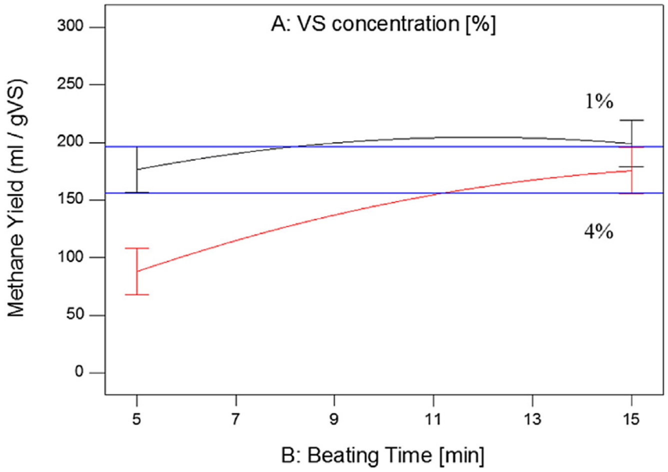

Fig. 12. *Laminaria* sp. *AB* interaction (single column).

matter during AD of *Ascophyllum nodosum* was the inhibitory effect of the polyphenols on methane production, while *Laminaria hyperborea* stipes were easily hydrolysed, since they contained much less polyphenols. It was reported that the content of polyphenols in *Ascophyllum nodosum* ranges between a maximum of 13% of dry matter during winter and a minimum of 9% in the summer [37,38]. While Schiener et al. [37] reported an average polyphenol content of only 0.15% of dry matter for both *Laminaria digitata* and *Laminaria hyperborea* and 0.41% for *Saccharina latissima*, being at high levels between May and July and low levels in October. In this experiment, *Ascophyllum nodosum* was harvested in August, while *Laminaria* sp. was harvested in May, thus it is likely that the polyphenol content was around 9% for *Ascophyllum nodosum* and around 0.2% for *Laminaria* sp. Such difference in polyphenols content could explain the more suitability of *Laminaria* sp. for methane production. This explains the best performance of *Ascophyllum nodosum* when the VS concentration was at the lowest level of 1% and the inhibition of methane production at the highest level of 4% of VS.

In the literature, few studies have compared these two brown species for biogas production. Hanssen et al. [22] carried out an AD of *Laminaria* sp. and *Ascophyllum nodosum* for a retention time of 30 days by investigating the VS concentration. In the case of *Ascophyllum nodosum*, it was recorded a methane production up to 140 ml g^{-1} VS at a VS concentration of 6.2%. The present work showed a methane yield from *Ascophyllum nodosum* in the same range (167 ml g^{-1} VS) at a lower VS concentration (1%) while an inhibition was observed at higher VS concentration of 4%. The methane yield measured at 4% of VS was less than half of the yields obtained by Hanssen et al. at 6.2% [22]. Hanssen et al. [22] did not consider the polyphenol content of *Ascophyllum nodosum*. However, considering that the harvesting times were close for both studies (September in Hanssen et al.'s study [22], August in the present work) it is likely that the content of polyphenols was quite similar. Nevertheless, the use of the beating pretreatment could explain the higher methane production at the lower VS concentration with respect to Hanssen et al.'s work [22]. The RSM analysis revealed that when the VS concentration was set at 1%, the pretreatment phase had a positive effect on the digestion as the methane production increased linearly with the time of pretreatment. The main effect of the beating pretreatment was to reduce the particle size of the substrate which allowed a better accessibility of the anaerobic microorganisms to the organic matter. Thus, according to these results, a VS concentration of 1% was sufficient in order to obtain methane production when the beating pretreatment was applied.

In the case of *Laminaria* sp., Hanssen et al. [22] registered up to 230 ml CH_4 g^{-1}VS at 5.8% of VS from *Laminaria hyperborea* and at 3.6% from *Laminaria saccharina*. The results reported in the present

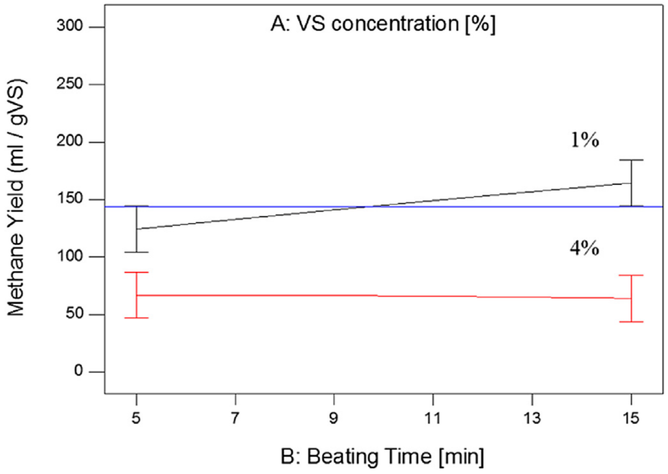

Fig. 11. *Ascophyllum nodosum AB* interaction (single column).

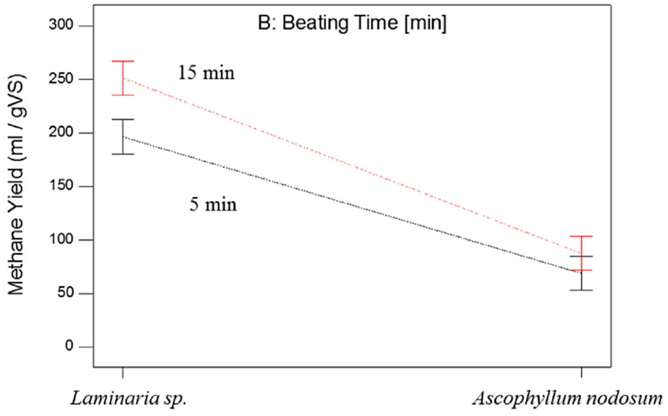

Fig. 13. *BC* interaction when *A* = 2.5% for *Laminaria* sp. and *Ascophyllum nodosum* (single column).

Table 9
Energy evaluation of optimisation (1) and (2).

	Optimisation 1: BT = 15 min	Optimisation 2: BT = 5 min
Methane yield [ml g^{-1} VS]	252	211
Methane content [%]	40	40
VS concentration [%]	2	2
Ep: Energy produced [Wh g^{-1} VS]	2.01	1.68
Ec: Energy consumed [Wh g^{-1} VS]	0.29	0.10
Net Ep [Wh g^{-1} VS]	1.72	1.59

work showed a methane production for *Laminaria* sp. in the same range (240 ml g^{-1}VS), but similarly to *Ascophyllum nodosum*, at a lower VS concentration of 2.5%. Thus, also for *Laminaria* sp., the pretreatment phase determined a more efficient digestion, as similar methane yields were reached at lower VS concentrations. Nevertheless, it must be noticed that Hanssen et al. [22] reported an initial failure of the digestion as a drop of pH (below 6.0) as well as high production of CO_2 were observed. Those were signs of an overloading of the digester, which was solved by adjusting the pH to 7.5, more suitable for the methanogenic population.

3.3. Optimisation

The first optimisation (1) problem was to find the optimal combination of seaweed species, VS concentration and beating time that could maximise the methane yield. The strategy of the software was to employ a desirability function (*d*) which varied between 0 and 1. When the response was at its goal, then *d* was equal to 1, on the contrary, when the response was outside an acceptable region, *d* was equal to 0 [35].

The software confirmed that when the aim was to maximise the methane yield, the best solution ($d = 0.913$) was to use around 2% of organic matter from *Laminaria* sp. and a beating pretreatment of almost 15 min.

A further optimisation (2) considered minimising the beating time while maximising the methane yield. In general, this combination is beneficial for the economics of the system as less energy is necessary for pretreatment.

In this case the highest desirability ($d = 0.787$) corresponded to employ *Laminaria* sp. after 5 min of pretreatment with a VS concentration of 2%.

It was noticed that in this optimisation the predicted methane response was 17% less than the previous optimisation in favour of a 10 min reduction of the pretreatment time. At this point, it was interesting to investigate if a reduction of 10 min in beating time could make up for a reduction of 17% of methane yield. Table 9 reports such analysis by employing the methane yields predicted by the software. The energy consumed [Wh g^{-1} VS] was calculated by measuring through a kilowatt hour meter, the electricity consumption of the machine at 15 min (0.12 kWh) and 5 min (0.04 kWh). The energy content of the biogas produced by the seaweed (B_S) was calculated equal to 3.99 kWh m^{-3} as mentioned in Section 2.7, by considering an average methane percentage of 40%. The analysis revealed that reducing the beating time of 10 min did not make up for a reduction of 17% of methane yield. The net energy at 15 min resulted to be 8% more energy output than the net energy produced at 5 min, according to the methane yields estimated by the software.

4. Conclusions

The objective of this experiment was to investigate the use of two indigenous Irish seaweeds, such as *Laminaria* sp. and *Ascophyllum nodosum* as feedstock for methane production through AD. An optimisation in terms of VS concentration and mechanical pretreatment was also carried out. The results concluded that *Laminaria* sp. was more suitable than *Ascophyllum nodosum* for biogas conversion, since a general enhancement of 40% in methane yield was observed. The RSM analysis highlighted that the VS concentration had a major impact on the methane yields of both species compared to the time of pretreatment.

It was observed that the *Ascophyllum nodosum* yields could be enhanced by optimising both the VS concentration and the beating time. In particular, the results showed that the highest methane yields were reached at 1% of VS and by increasing the beating time up to 15 min. A general 30% more methane was achieved with respect to the untreated sample.

Laminaria sp. exhibited the highest methane yields when the VS concentration was set at 2.5% and after 15 min of beating treatment. In this case, only a marginal improvement with respect to the untreated sample was observed, even though results at 6 days of digestion revealed an enhancement of methane yields of more 50% with respect to the untreated sample. Thus, it was likely that in this case the major effect of the pretreatment was an acceleration of the digestion process. This trend was not observed in the case of *Ascophyllum nodosum.*

Acknowledgements

This research was supported by the Irish Research Council for Science, Engineering and Technology, funded by the National Development Plan (RS/2012/417).

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