Improvement of methane production from *P. canaliculata* through mechanical pretreatment

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Abstract

In order to increase the methane yield from biomass, this paper investigates the mechanical pretreatment effect of a Hollander beater on the anaerobic digestion process of *Pelvetia canaliculata* macroalgae using a response surface methodology (RSM). Higher values on methane yield were obtained at lower F/I ratios, with an increase up to 2.5-fold at an F/I value of 0.3. A multi-objective optimization study was carried out with the aim of maximizing the methane yield while minimizing the pretreatment time. An optimum methane yield of 283 ml/gVS was obtained for 50 min pretreatment time and a ratio F/I of 0.3, which represents an increase of 45% compared to non-pretreated algae.

Keywords: renewable energy, biomass, biogas, algae, mechanical pretreatment, anaerobic digestion

Abbreviations: AD, anaerobic digestion; ANOVA, analysis of variance; BT, beating time; DT, digestion time; CCD, central composite design; F/I, feedstock/inoculum; KDP, potassium dihydrogen phosphate; MC, moisture content; NB, no beating; RSM, response surface methodology; TS, total solids; VFAs, volatile fatty acids; VS, volatile solids.

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1 INTRODUCTION

Macroalgae biomass production is currently a growing industry used in fertilisers, food, medicines, chemicals production and as a source of bioenergy [1,2]. Bioethanol, biogas and biodiesel can be obtained from macroalgae by different conversion methods including fermentation, anaerobic digestion and transesterification [3,4]. Macroalgae biomass for bioenergy production first received attention in 1973 as part of the US Ocean Food and Energy Farm project and resulted in the construction of ocean farms for cultivation of the giant seaweed *Macrocystis* [5]. Macroalgae, unlike first generation biomass, do not compete with food crops and do not need large areas of arable land and fresh water resources, also their low lignin content makes their biodegradation easier compared to lignocellulosic biomass. They can be cultivated on longline systems, similar to those used for mussel production, in vertical or horizontal kelp rope systems [5,6].

Methane production from macroalgae is related to ash content and the level of storage sugars. It varies with biochemical composition and usually range from 120 to 480 ml/gVS [3]. Before undergoing anaerobic digestion, macroalgae should be suitably conditioned in order to offer a larger target surface area to the microorganisms in the digester and thus to improve and accelerate the degradation process [7–9]. The availability of the substrates for the enzymatic attack will be achieved through the increment of the specific surface area and breakdown the macroalgal structure. In recent years different technologies for biomass pretreatment have been developed in order to increase the availability of substrate for anaerobic digestion [10,11]. Maceration of *Gracilaria vermiculophylla* improved its methane yield by 12% from 430 ml/gVS to 481 ml/gVS, further thermochemical pretreatment using NaOH increased the algae solubilisation but not its methane yield [12]. Reducing the particle size of *Rhizoclonium* with a warring blender from 1cm to 0.1mm increases the methane yield by 13%. The blended algae were then submitted to biological pretreatment with five different enzymes: lipase, xylanase, a- amylase, protease and cellulose; protease addition was the less effective with a methane yield of 116 ml/gTS while a combination of the five enzymes achieved a methane yield of 145 ml/gTS which means a 21 % extra methane yield compared to blended algae [13]. A pressure pretreatment using a prototype machine owned by TK Energi broke up the structure of *Fucus vesiculosus* into a homogenous slurry and improved the methane yield from 67 ml/gVS to 92 ml/gVS [14].

In this paper, an investigation of a mechanical pretreatment, named beating [15], of *Pelvetia canaliculata* macroalgae was reported. A parametric analysis has been carried out to investigate the effect of beating time and feedstock/inoculum (F/I) ratio on the methane yield. Furthermore, a multi-objective optimization was conducted with the aim of maximizing the methane yield while minimizing the pretreatment time.

2 MATERIALS AND METHODS

2.1 Feedstock and inoculum

Macroalgae were collected on-shore in Rothesay (Isle of Bute, Scotland) in March 2016 and used unwashed within 48 h. *P. canaliculata,* commonly known as Channelled wrack, is perennial brown seaweed characterized by a 15 cm-long dichotomous, narrow thallus with folded gutter-like branches (Figure 1). Living for about 4 years at the upper limit of the intertidal zone, populations of *P. canaliculata* are found on the Atlantic rocky shores of Europe from Iceland to Spain [16].

Figure 1. Raw algae (left) and 30 min pretreated algae (right).

The sludge used as inoculum was provided by the Energen Biogas Plant (Cumbernauld, Scotland) using food processing residues as feedstock, stored in a fridge at 4 °C and used within 48 h. The total solids (TS) and volatile solids (VS) of the sludge and the macroalgae were calculated by duplicate and were obtained by submitting random samples to105 °C (for TS) and 550 °C (for VS) until constant weight. The VS are expressed as percentage of TS. The methane production is provided in terms of volume per gram of VS (ml/gVS). The characterization of the algae and the sludge is detailed in Table 1.

Table 1. Algae and sludge characterization.

Parameters	Inoculum	Untreated algae	30 min pret. algae	60 min pret. algae
Total Solids (%)	4.70 ± 0.01	18.7 ± 0.01	6.04 ± 0.01	6.02 ± 0.01

Volatile Solids (%)	62.98 ± 0.09	81.68 ± 0.06	81.68 ± 0.06	81.68 ± 0.06
Ash content (%)	37.02 ± 0.09	18.32 ± 0.06	18.32 ± 0.06	18.32 ± 0.06

2.2 Hollander beater pretreatment

The pretreatment process was conducted using a modified Hollander beater. This beater is normally used in the paper industry for pulping [17]. However, most of mechanical biomass pretreatment processes can use existing facilities previously designed for other purposes and other materials with minor modifications. This is considered as a significant advantage for biomass mechanical pretreatment compared to other types of pretreatments [8,18].

Hollander beater is made entirely of non-corrosive materials, the beater used in this study is made of stainless steel, and it is composed of an oval vessel divided along its major axis by a partition that do not reach the walls, so an elliptic channel is formed. In one of the sides of the channel a bladed drum is placed spinning at 580 RPM above a bedplate churning pulp up over the back fall where it slides down creating momentum to round the curve and continue the loop. The feedstock is exposed to the shear action of the rotating bars against the bedplate. The biomass should be soaked prior its treatment in the beater; in this study, the algae samples were fresh and there was no need to soak them before the pretreatment [19–21]. The capacity of the beater is about 900 g of dry biomass, but this can vary depending upon the type of feedstock. Samples were taken after 30 and 60 minutes of beating pretreatment. The samples were taken from the bend before reaching the bladed drum to take the most representative sample.

2.3 Bioreactors

The bioreactors consisted of 500 ml Erlenmeyer flasks with working volume of 400 ml connected through a system of valves and pipes to airtight bags (Linde Plastigas) for biogas collection. To clear up any trace of oxygen from the system and preserve the anaerobic conditions, nitrogen was flushed into the headspace of each reactor. The reactors were placed in a water-bath to keep the bioreactors at the mesophilic temperature of 37°C.

Reactors were fed with a fixed amount of 200 ml of sludge (inoculum), while different quantities of pulp (beated algae) were required to set the F/I ratio at 0.3, 0.5 and 0.7. The pH was adjusted to 6.70±0.15 with potassium dihydrogen phosphate (KDP) as a buffer solution and measured by a pH meter Corning model 120. The reactors corresponding to the untreated samples were feed with fresh algae. In order to assess the inoculum contribution to the methane production, control batches were prepared in the same way except for the algae addition. Flasks were daily shaken during the process in order to facilitate the degasification of the substrate and the contact between the biomass and the inoculum. Each test was conducted in duplicate, and the average results are reported in this paper.

For gas volume measurement, a graduated upside-down cylinder that is connected to a bubbling flask was used in order to maintain the necessary oxygen-free conditions and to avoid air infiltrations. The methane content was test with a gas analyser (Drager X-Am 7000). The digestion was stopped when the methane production rate was found to be less than 1% of the overall volume produced [22]. The methane volumes are given for a dry gas in standard conditions of temperature (0°C) and pressure (1 atm).

2.4 Design of experiments

The experiment was planned according to a response surface methodology (RSM) for two factors, beating time and F/I ratio with three levels. The response was the methane production per g of VS of added macroalgae. RSM consists of a group of mathematical and statistical techniques used in the development of an adequate functional relationship between a response of interest, *y*, and a number of associated control (or input) variables denoted by $x_1, x_2, \ldots, x_k$. Usually, a second order polynomial as shown in Equation 1 is used in RSM to describe this functional relationship.

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_{ii}^2 + \sum b_{ij} x_i x_j \quad (1)$$

where the values of the model coefficients b_0, b_i, b_{ii} and b_{ij} are estimated using regression analysis [23,24]. In this study, the RSM was applied through a central composite design (CCD) to fit a model by least squares technique.

Factor levels of independent input variables are respectively 0, 30 and 60 min for the beating time BT and 0.3, 0.5 and 0.7 for F/I ratio.

The adequacy of the models is tested through the analysis of variance (ANOVA). The statistical significance of the model and of each model term is examined using the sequential F-test and lack-of-fit test. If the Prob. > F of the model and of each term in the model does not exceed the level of significance (in this case $\alpha = 0.05$) then the model may be considered adequate within the confidence interval of $(1 - \alpha)$. An adequate model means that the reduced model has successfully passed all the required statistical tests and can be used to predict the responses or to optimize the process. The values of R^2, adjusted-R^2, predicted-R^2, lack of fit and adequate precision of models are obtained to check the quality of the suggested polynomial. The response surface model and the subsequent statistical study was performed using the Design Expert software version 9.

3 RESULTS AND DISCUSSION

3.1 Methane production

The methane production from *P. canaliculata* after beating pretreatment is shown in Figure 2. The inoculum contribution of methane production was subtracted from final figures and it was never higher than 10%. The methane production from 200 ml of inoculum (control batch) on day 7 was 23.28 ml, on day 14 was 38.80 ml and on day 21 was 46.56 ml.

The beated samples achieved higher methane yields compared to the respective untreated samples, approximately 74% for the algae beated for 60 min, and 6% for samples beated for 30 min. Beating pretreatment increased the surface area of the biomass making it more accessible to the microorganisms, providing higher biodegradation rates and facilitating a fast hydrolysis. For a ratio F/I 0.3, the methane production increase was most noticeable at early stages of the degradation. On day 7, the methane production from 60 min treated samples was 112% higher than the untreated samples, and on day 14 was 78%. For ratios F/I of 0.5 and 0.7, the effect of pretreatment was much less noticeable. It can also be noticed that by increasing F/I ratio and decreasing the digestion time (DT), the effect of the pretreatment decreases. For a ratio of 0.5, on day 14 of digestion the methane production from 60 min treated samples was 62% higher compared to untreated samples. For the same DT and BT and a ratio F/I 0.7, the increase on methane production was 19%. The methane production increase between treated and untreated samples was more significant at later stages of digestion than at start of the process (Figure 2).

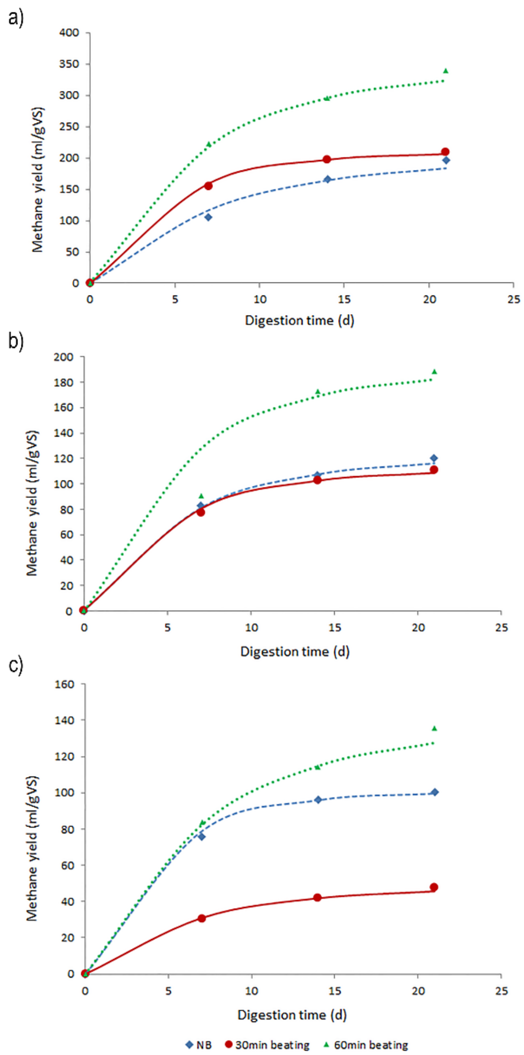

Figure 2. Methane production for different pretreatment times: a) ratio F/I 0.3, b) ratio F/I 0.5 and c) ratio F/I 0.7.

3.2 Experimental Design

The experiment parameters, BT and F/I were checked in three levels. Beating time varied between 0 and 60 minutes while ratio feedstock/inoculum varied between 0.3 and 0.7. The response was set as the methane production given in ml per g of volatile solids (ml/gVS). Parameters and results are shown in Table 3.

Table 2. Experimental factors and responses for the methane model estimation

Experiment number	Experimental factors		Response
	Beating time (min)	Ratio F/I	Methane yield (ml/gVS)
1	0	0.5	120 ± 15
2	30	0.3	208 ± 05
3	30	0.5	110 ± 09
4	30	0.7	40 ± 08
5	0	0.3	196 ± 21
6	60	0.3	340 ± 40
7	60	0.7	135 ± 12
8	30	0.5	110 ± 09
9	0	0.7	100 ± 05
10	60	0.5	188 ± 30

For the modelling of the methane production through the RSM, the model F-value of 100.92 implies the significance of the model. There is only a 0.03% chance that an F-value this large could occur due to noise. The model terms of R^2 = 0.9921, adjusted-R^2 = 0.9823, predicted-R^2 = 0.9329, all these values are very close to 1 and the adjusted-R^2 and the predicted-R^2 are within 0.2 indicating the adopted model is adequate. The adequate precision, which measures the signal to noise ratio, is 33.059. A ratio greater than 4 indicates an adequate signal and the model can be used to navigate the design space.

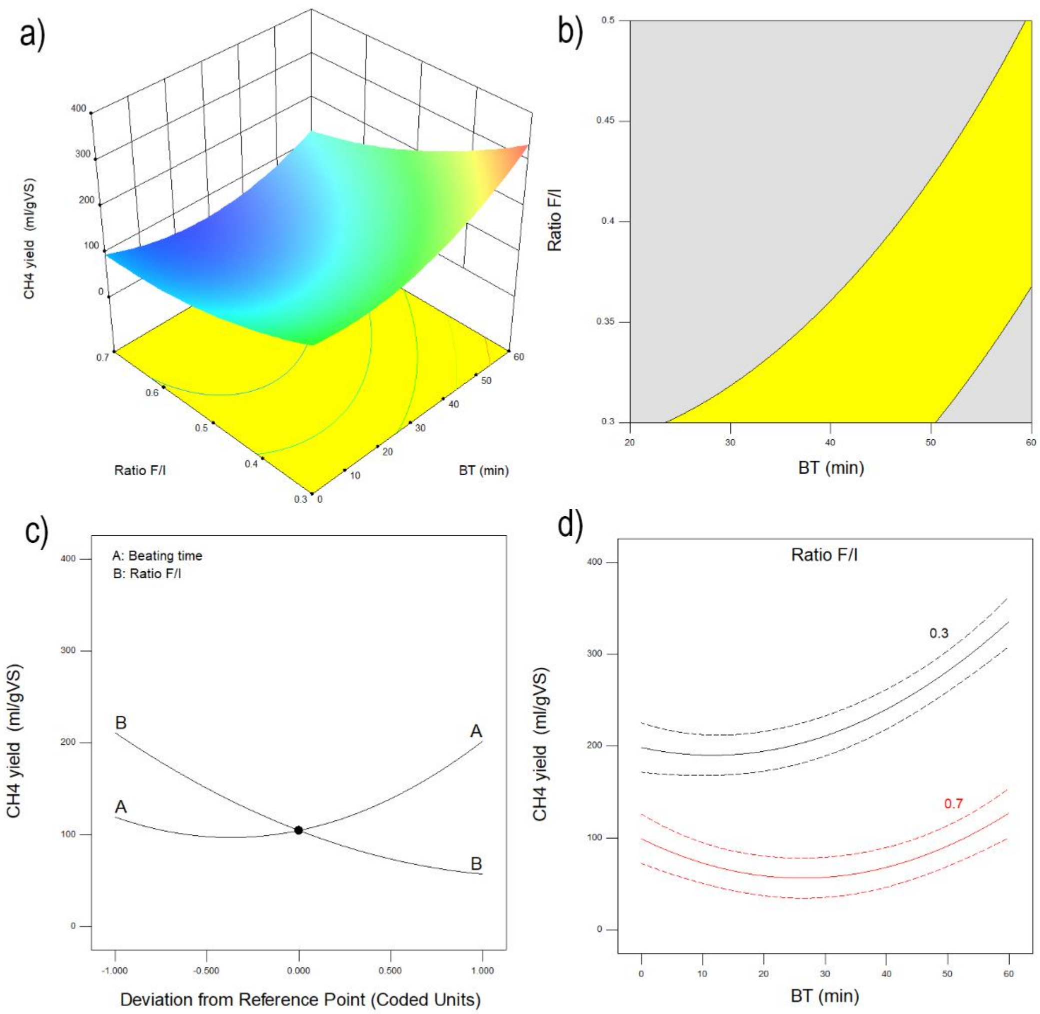

Figure 3. Response surface plots for methane production in 3D (a), graphical optimization (b), perturbation plot (c) and interaction plot (d).

The response surface obtained from the model illustrated in Figure 3a shows that higher methane yields were obtained at higher beating times and at lower F/I ratios. The methane yield was found to increase with decreasing F/I ratio, both for treated and untreated samples. The methane yield for untreated algae at 0.3 F/I ratio was 196 ml/gVS and at 0.7 F/I ratio was 100 ml/gVS. According to the guideline "Fermentation of organic materials" [22], the optimum F/I ratio is 0.5. This study shows that this ratio can be reduced to 0.3 for the anaerobic digestion of macroalgae. Knowing the optimum F/I ratio allows a better exploitation of the feedstock. Feeding the reactor with high quantities of biomass that the inoculum is not able to process leads to a loss of feedstock, hence is not digested. Increases of 15-61 % in the methane production rate constant were observed in the beated samples for F/I ratio 0.3 (Table 3). For a ratio of 0.5, the methane production rate constant increases by 12.5 % for 30 min beating and did not vary for 60 min beating. For a ratio of 0.7, the methane production rate constant decreased with increasing beating time.

At the end of the incubation period, with a 50 % of methane content, the biogas yield was 356.36 ml/gVS. This result was higher than the value of 196.39 ml/gVS reported by Tedesco et al. (2013), but no F/I ratio was mentioned. However the result from Tedesco et al. agreed with the biogas production achieved in the present studio at a F/I ratio of 0.5, 220.38 ml/gVS [25]. An excessive particle size reduction of the substrate accelerates the hydrolysis and acidogenesis in the early stage of anaerobic digestion, resulting in accumulation of volatile fatty acids (VFAs) that leads to a decrease in pH, causing a decrease in methane production [8,26]. The final pH in this study remained constant around a value of 6.78±0.15 similar to the initial pH. These values did not suggest the occurrence of any strong inhibition due to VFA accumulation.

The perturbation plot in Figure 3c shows how the methane yield is affected by the input variables beating time and F/I ratio. Both variables have an exponential effect on the methane production. By increasing A (beating time) the methane yield will increase exponentially, meanwhile increasing B (F/I ratio) the methane yield will decrease also exponentially. The effect of pretreatment is observed to be more important at low F/I ratios as can be displayed from the interaction plot (Figure 3d), for an F/I ratio of 0.5, the methane yield improved exponentially with the beating time from a minimum of 12 min. For a F/I ratio of 0.7, the minimum methane yield was achieved at higher beating time (25 min) and then improved until reach a value around 120 ml/gVS.

To verify the adequacy of the developed models, three confirmation experiments were carried out using new test conditions which are within the experimental range earlier defined. The experimental conditions, the actual and predicted values and the percentages of error are summarized in Table 4. Considering that anaerobic digestion is a biological process, the percentages of error are all within acceptable tolerances.

Table 3. Validation experiments

Experiment	Beating time (min)	Ratio F/I		Methane yield (ml/gVS)
			Actual	130.38
1	20	0.4	Predicted	137.53
			Error (%)	-5.48
2	35	0.6	Actual	80.73

			Predicted	77.16
			Error (%)	4.42
			Actual	258.03
3	45	0.3	Predicted	254.98
			Error (%)	1.18

Based on the response surface model, a multi-objective optimization study was conducted using the desirability approach to evaluate the best combination of each of the process parameters that results in the best process output, as judged based on a number of specific practical criteria. Solving multi-objective optimization problems using the desirability approach consists of a technique that combines multiple responses into a dimension-less measure of performance, called an overall desirability function. Each process parameter and response can be assigned an importance relative to the other parameters and responses. Importance varies from the least important value of 1 (+), to the most important value of 5 (+++++). The optimization study by means of the desirability approach was conducted using Design-expert v9 software. The numerical optimization provides the ideal input variables levels to achieve the highest methane yield and the graphical method results in a plot that associates the input variables levels to an area of target outputs defined by the user.

The optimization criteria combine the productivity with the cost of the process. The aim is an effective treatment of the biomass (maximizing the algae digestibility) while minimizing the energy consumption of the pretreatment method. For the optimization, methane production was maximized with level 5 and beating time was minimized with level 1 while F/I ratio was permitted to vary in the same range as seen in Table 3.

The optimal methane production (283 ml/gVS) from the numerical optimisation was found at BT= 50 min and F/I ratio= 0.3, allowing 45% extra methane when compared to the maximum methane production for untreated algae.

The graphical optimization allows a selection of the optimum process parameters by means of visual inspection. The grey areas on the overlay plot (Figure 3b) represent the values that do not meet the proposed criteria; the target area in yellow is delimited by the curves corresponding to the optimization criteria set by the authors. The lower and upper limits were chosen according to the numerical optimization results, 198 ml/gVS and 283 ml/gVS.

4 CONCLUSIONS

The experimental work shows the effect on the methane production from macroalgae of a new mechanical pretreatment. The methane production of *P. canaliculata* increased by 74% when the algae was beated for 60 min, from a value of 196 ml/gVS correspondent to untreated algae to 340 ml/gVS. A pretreatment time of 30 min resulted in a methane yield of 208 ml/gVS. Higher methane yields were achieved at an optimum F/I ratio of 0.3, with a maximum increase of 2.5-fold compared to an F/I ratio of 0.7. An optimization study was performed to reduce the operating costs associated to the pretreatment and to maximize the productivity. The optimization criteria was set for a maximized methane production while minimizing the pretreatment time. An optimum methane production of 283 ml/gVS was obtained for a 50 min beating pretreatment and an F/I ratio of 0.3. The study demonstrated the Hollander beater pretreatment increased the methane production of the macroalgae *P. canaliculata* through anaerobic digestion. Current results suggest that Hollander beater may be applied to others lignocellulosic feedstock for an improved digestibility.

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