Tedesco, Silvia, Marrero Barroso, T and Olabi, AG (2014) Optimization of mechanical pre-treatment of Laminariaceae spp. biomass-derived biogas. Renewable Energy, 62. pp. 527-534. ISSN 0960-1481

DOI: https://doi.org/10.1016/j.renene.2013.08.023

Publisher: Elsevier

Version: Accepted Version

Optimization of Mechanical Pre-Treatment of *Laminariaceae* spp. Biomass-Derived Biogas

S. TEDESCO[*], T. MARRERO BARROSO[a], A.G. OLABI[b]

[*] Department of Mechanical and Manufacturing Engineering, Dublin City University, Glasnevin, Dublin 9, Ireland. Email: silvia.tedesco3@mail.dcu.ie

[a] Department of Electronic Engineering, Dublin City University, Glasnevin, Dublin 9, Ireland.

[b] Institute of Energy and Engineering Technology, University of the West of Scotland, Paisley,PA1 2BE, Scotland; email: abdul.olabi@uws.ac.uk

Abstract

Macroalgae have not met their full potential to date as biomass for the production of energy. One reason is the high cost associated with the pretreatment which breaks the biomass's crystalline structure and better exposes the fermentable sugars to anaerobes. In the attempt to overcome this technological barrier, the performance of a Hollander beater mechanical pretreatment is assessed in this paper. This pretreatment has been applied to a batch of *Laminariaceae* biomass and inoculated with sludge from a wastewater treatment plant. The derived biogas and methane yields were used as the responses of a complex system in order to identify the optimal system input variables by using the response surface methodology (RSM). The system's inputs considered are the mechanical pretreatment time (5-15 minutes range), the machine's chopping gap (76-836 μm) and the mesophilic to thermophilic range of temperatures (30-50 °C). The mechanical pretreatment was carried out with the purpose of enhancing the biodegradability of the macroalgal feedstock by increasing the specific surface area available during the anaerobic co-digestion. The pretreatment effects

Abbreviations: AD, Anaerobic digestion; ANOVA, Analysis of Variance; BBD, Box-Behnken design; BT, Beating Time; COD, Chemical Oxygen Demand; HRT, Hydraulic Retention Time; MC, Moisture content; MG, Machine's gap; RSM, Response Surface Methodology; T, Temperature; TS, Total Solids; VFA, Volatile Fatty Acids; VS, Volatile Solids.

on the two considered responses are estimated, discussed and optimized using the tools provided by the statistical software Design-Expert v.8. The best biogas yield of treated macroalgae was found at 50 °C after 10 minutes of treatment, providing 52% extra biogas and 53% extra methane yield when compared to untreated samples at the same temperature conditions. The highest biogas rate achieved by treating the biomass was 685 cc gTS^{-1}, which is 430 cc gTS^{-1} in terms of CH_4 yield.

Keywords: Anaerobic Co-digestion; *Laminaria spp.*; Sludge; Mechanical Pretreatment; Methane yield; Optimisation.

* Corresponding author: Department of Mechanical and Manufacturing Engineering, Dublin City University, Glasnevin, Dublin 9, Ireland. E-mail address: silvia.tedesco3@mail.dcu.ie. Tel.: +353 1 700 7563

[a] Email: tony.marrero@eeng.dcu.ie

[b] Email: abdul.olabi@uws.ac.uk

1 Introduction

Brown macroalgae have been largely investigated for bioenergy production [1], because of their special characteristics like absence or very low lignin content, high carbohydrates and low lipid levels. All these advantages make their anaerobic biodegradation easier than their relatives' microalgae. In fact, because of their lipid cell walls, microalgae usually require high-pressure pretreatments in order to obtain their cell disruption [2]. Besides biogas [3, 4], numerous pioneering studies have been undertaken on *Laminaria* spp. as bio-feedstock to produce other varieties of biofuels; special attention has been paid to bioethanol [5-10] and biohydrogen [11-13] production. Very recently Qin et al. [14] pushed further the frontier of sustainability by genetically engineering algae in order to increase their growth rate to meet the growing demand for algal biofuels, and thus address the challenge of the biomass feedstock supply. The scope of the study was to also modify the microalgae's metabolic pathway for a more efficient production of high-value products. The natural fast growing rates of brown macroalgae also led to very interesting studies on marine biomass aquaculture for CO_2 fixation [15-16] in which the macroalgae's remediation potential has been identified. Biogas itself has been found to mitigate greenhouse gas emissions when used as transport fuel [17].

Biogas can be derived via anaerobic fermentation of any organic matter, including the cellulose and hemicellulose within plants, although the biomass must be subjected to pretreatment processes in order to liberate the sugars needed for fermentation [18, 19]. In the literature many types of pretreatments have been used, depending on the substrate's morphology, to perform different tasks while consequences to methane production were estimated [20-24]. A mechanical pretreatment phase is usually the first step not only for methane but also for bioethanol fermentation [25]. In particular, milling, grinding and extrusion can be accounted amongst the most successful mechanical pretreatments on lignocelluloses to biogas production. In fact, studies conducted on milling [20], revealed this technique is effective at increasing the methane by 5-25% for most lignocelluloses, without producing inhibitors. For some lignocelluloses, such as sisal fibre waste, the methane yield improvement is even higher and can reach from 31% up to 70% [26]. Silva et al. [27] have studied the effect on enzymatic degradation of wheat straw using ultra-fine grinding by ball-milling and air-jet milling. Ball-milling appeared to be the most effective pretreatment by enhancing the total carbohydrate degradability up to 46% and the glucose hydrolysis yields up to 72%. Bead mill effects on methane yields from food waste were investigated by Izumi et al. [28]. Findings reveal that methane improvement of 28% occurred when compared to untreated fibers. Finally, Hjorth et al. [29] have found that methane production from deep litter is enhanced by 9-70% after 28 days when the extrusion is used as pretreatment. In general, all these mechanical pretreatment techniques are high energy demanding [30-33]. The high pretreatment's cost has been in fact identified as one of the key barriers for commercialization of lignocellulosic biofuels [34]. In order to help overcome this technological obstacle and make seaweed exploitation to bioenergy economically feasible, a Hollander beater pre-treatment's effects on biogas and methane yields from indigenous Irish *Laminariaceae* sp. have been investigated in this paper. This technique is based on the same 'comminution' concept proposed by all other mechanical treatments and has been applied to macroalgal feedstock, achieving promising results [35]. A co-digestion with digester sludge is used to provide the necessary bacteria in the digesting reactors. The response surface methodology (RSM) was applied in the experiment and aims to identify the ideal levels of pretreatment parameters and digesting temperature which will result in the best biogas and methane yield. Finally, an operating cost minimization analysis was carried out by using the optimisation tools of the statistical software Design-Expert 8.0.

2 Materials and methods

2.1 Pre-treatment machine

The pre-treatment machine consists of a modified Hollander beater; model Reina as shown in Fig. 1. The machine's gap between the blades and bed-plate is adjustable by using a crank handle. A single turn of the crank handle corresponds to 76µm. The grooves located on the bed-plate exercise a cutting action while the high pressure and speed reached under the drum beat the mixture, creating the macroalgal pulp used to feed the reactors. This machine can be used to treat dry and wet biomass, but it requires the addition of water to the substrate in order to recirculate the stock.

2.2 Co-digesting feedstock and inoculum

The seaweeds batch was collected on-shore in Howth (Dublin, Ireland) in early June for the response surface methodology (RSM) experiment, and was treated and inoculated the same day. The *Laminarinaceae* spp. identified in the batch were *L. Digitata* mainly, *L. Saccharina (Saccharina Latissima)* and *L. Hyperborea*.

Sludge was used as inoculum and collected in the wastewater plant of Ringsend (Celtic Anglian Water Ltd.), Dublin, Ireland. Such inoculum was not allowed to degasify in order to simulate the real operating conditions of a co-digestion facility. Hence, the sludge contribution in terms of biogas and methane volume rates was deducted from the co-digesting yields. In order to estimate such sludge contribution, a reactor in double replication has been used to ferment sludge-only at each digesting temperature of the RSM model. A tank of sludge was collected from the plant the same day of the experiment and used immediately. As the sludge composition changes on a daily basis, the full RSM experiment used sludge with characterization shown in Table 1.

2.3 Batch bioreactors preparation

The bioreactor system consists of flasks of 500 ml in capacity each. The equipment is constituted of: 2-way and 3-way valves, quick release tubing connectors, plastic pipes and airtight plastic bags for biogas collection; see Fig. 2. The anaerobic conditions are created by purging nitrogen in the system for 2 minutes according to procedure VDI 4630 [36]. Water-baths were used to keep the reactors at the desired temperature within an interval of confidence of ±1°C. During the incubation, flasks were gently shaken every 20 hours in order to favour the degasification of the substrate and the contact between the biomass and the inoculum. When the biogas production rate was found to be less than 1% of the overall volume produced, the digestion was stopped according to [36]. Hydraulic retention time (HRT) was 21 days. 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 gas collection.

2.4 Experimental Methodologies

The experiment aims to find the optimal levels of the Hollander beater’s operating parameters such as the beating time (BT) of treated biomass and the ideal machine’s gap (MG) combined with fermentation temperature (T), while assessing how such parameters affect the biogas yield and its methane content. Due to a limited capacity of water-baths, a design of experiment (DOE) was performed in order to minimize the number of reactors needed for the experiment. The pretreatment machine operated on a mixture of 2 kg of on-shore biomass (fronds) and 20 litres of water. Reactors of untreated samples contain 30 g of wet plant and 300 ml of sludge, while treated reactors contain 200 ml of macroalgal pulp with 300 ml of inoculum. The moisture contents of both untreated seaweeds and macroalgal pulp were assessed to provide a comparison of biogas and methane yield per gram of total solids (TS = 1 – moisture content (%)), see Table 2. TS of the untreated frond were 14.4%. The drying temperature was 105°C, until constant weight was achieved.

2.5 Design of Experiment

The response surface methodology (RSM) adopted for the second experiment follows the Box-Behnken Design (BBD) whose variables are shown in Table 3. This methodology was then applied to the measured yields using the statistical software, Design-Expert v.8. RSM is a set of mathematical and statistical techniques that are useful for modelling, interpreting and predicting the response of interest to several input variables χ (from levels i to j) with the aim of optimizing a single or multiple response "y_s". The independent variables in this study are the pretreatment time (BT), fermentation temperature (T) and the machine's gap (MG). The second order polynomial model, given by equation (1), was fitted using a step-wise regression (α=0.01) via Design-Expert v.8 and it was applied on two responses (y_1, y_2): the biogas and methane yields per gram of total solids (cc gTS^{-1}). The same statistical software was used to generate the analysis of variance (ANOVA) and the response plots.

$$Y = b_o + \sum b_i \chi_i + \sum b_{ii} \chi_{ii}^2 + \sum b_{ij} \chi_i \chi_j \qquad (1)$$

The values b_0, b_i, b_{ii} and b_{ij} represent the regression coefficients. The *p*-value of the model has been computed using α=0.01 so that the model may be considered adequate within the confidence interval of $(1-\alpha)$ [37]. Results are then used to run an optimization study using the numerical and graphical methods provided by Design-Expert in order to find out the best factor levels that, under specific user-defined criteria, will maximize the system's responses.

3 Results and Discussion

3.1 Model estimation

The RSM provided the optimum combinations to be tested in order to capture the biggest variability in y_S with the minimum amount of runs. Table 4 shows the results of biogas and methane yield according to the RSM coded design matrix, sorted by standard order. The fit summary output indicates that the quadratic model is statistically significant for both responses. A reduced quadratic model analysis was adopted for both responses resulting in the model terms of $R^2 = 0.9372$, adjusted-$R^2 = 0.9196$, predicted-R^2 0.8641, adequate precision = 21.188 for the biogas yield, and $R^2 = 0.8536$, adjusted-$R^2 = 0.8126$, predicted-R^2 0.6383, adequate precision = 15.799 for the methane yield. The values of R^2, adjusted- R^2 and predicted- R^2 are very close to 1 and so indicate the adopted model is adequate. The achieved adequate precision is >> than 4, which indicates good model discrimination. The residuals are shown in Fig. 3A and 3B respectively for biogas and methane yields. Since the internally studentized residuals are reasonably close to the normal probability diagonal, these Fig.s indicate that the developed models are adequate and fit the data with a normal distribution of probability.

The analysis of variance (ANOVA) indicated that the temperature (T), the machine's gap (MG), the two level interactions of (T x MG), and the second order effects of (T^2), beating time (BT^2) and (MG^2) are the most significant factors affecting the biogas yield. The most important factors affecting the methane yield instead are: MG, (T x MG) and T^2 and

MG^2. The final mathematical model associated to the responses in terms of coded factors (eqs. (2) and (3)) and actual factors (eqs. (4) and (5)) determined by the software are shown below.

$$\text{Biogas Yield} = +157.92 - 89.92\ T + 23.77\ BT - 85.55\ MG - 46.40\ T\ MG + 265.17\ T^2 - 69.23\ BT^2 + 119.60\ MG^2 \quad (2)$$

$$CH_4\ \text{Yield} = +114.38 - 3.55\ T + 15.39\ BT - 48.58\ MG - 45.87\ T\ MG + 95.62\ T^2 - 34.85\ BT^2 + 73.81\ MG^2 \quad (3)$$

$$\text{Biogas Yield} = +4474.22 - 216.49\ T + 55.38\ BT - 26.83\ MG - 92.80\ T\ MG + 2.65\ T^2 - 2.53\ BT^2 + 4.78\ MG^2 \quad (4)$$

$$CH_4\ \text{Yield} = +1428.85 - 72.34\ T + 30.74\ BT - 2.54\ MG - 0.9175 T\ MG + 0.96\ T^2 - 1.39\ BT^2 + 2.95\ MG^2 \quad (5)$$

The perturbation plot in Fig. 4A shows that factors T and MG affect the biogas volume response in a convex way, while BT affects it in a concave way. This suggests the following: i) decreasing the temperature from 40°C to 30°C or increasing it from about 40°C to 50°C has a consistent positive effect on the biogas yield; ii) the desirable setting of the gap is 0 turns; iii) BT increase is beneficial up to the vertex of the effect’s curve (at about 11 minutes), after this point prolonging the treatment time results in a negative effect on the biogas production. Fig. 4B illustrates that a significant interaction may occur between T and MG when the temperature is around 33.8°C, at the intersection of the confidence bands. This means that when incubating at $T \leq 33.8$°C, a tighter gap would be recommendable. The perturbation behaviour of the methane response is plotted in Fig. 5A. Factors T and MG have again a convex effect, while BT has a concave one. In the case of methane production, the desirable temperatures are around either 30° or 50 °C, and using a gap larger than -1 (0 turns) will result in a significant CH_4 decrease. The same considerations made for BT effect on the biogas yield apply to the methane response. The interaction plot in Fig. 5B shows that incubation at 50°C is preferable at $MG\leq5$; otherwise a digesting temperature of 30°C should be used in order to maximize the methane yield. The combined role played by BT and MG is quite interesting on the mechanical treatment effectiveness's point of view. Basing on the perturbation-interaction plots and the results in Table 4, it appears that certain level combinations of these two factors (in the region where $5\leq MG\leq10$ and $10\leq BT<15$) interact in such a way that both yields result reduced. This is particularly visible in the methane production. The interaction plots in Fig.4B and Fig. 5B suggest that a methanogenic inhibition takes place around 40°C. Methane yield reduction of other mechanically over-treated substrates was observed in the literature and it seems due to inhibitory phenomena development during the digestion. These can be caused by multiple factors, the main ones are the accumulation of VFAs and consequent pH alteration, the accumulation of long chain fatty acids (LCFAs) or NH_4^+, and the production of H_2S [38]. The response surface so obtained is shown in Fig. 6A for the biogas yields and Fig. 6B for the methane yield.

3.2 Biogas and methane production

Across the period examined, the digester sludge contribution to the overall biogas formation was 930, 1030 and 100 cc respectively at 50°C, 40°C and 30 °C with a CH_4 content of about 30% in each case. The sludge's biogas and methane production has been subtracted from the co-digesting results in order to determine the substrate's contribution. Samples of untreated material produced an average of 332, 305 and 319 cc gTS^{-1} of biogas respectively at 50°C, 40°C and 30 °C. The methane content peaked at 40°C where it reached the value of 51%, while it slightly decreased from 47% to 44% when passing from 50°C to 30°C. The results of the treated samples are provided in Table 4, while Fig. 7 shows the mean biogas (A) and methane (B) yields achieved during the RSM experiment, sorted by standard order and correlated to their standard deviations. Assuming an ash content according to [39], it can be seen that most of the methane yields in Table 4 are in range with those found in the literature at about the same digesting temperature and for the same period of the year [40-42], but the thermophilic range seems to offer better volumes. Thermophilic temperatures were also found to improve the conversion of microalga *Spirulina Maxima* to methane [43]. In fact, the best result of both biogas and methane yield were achieved at 50°C when using BT=10 minutes and MG=0 as the pretreatment settings (samples 6 and 23). These settings allow up to 52% extra gas and 53% extra methane yields when compared to the untreated substrate at the same temperature. Nevertheless, a thermophilic range of temperature has been found to make the fermentation unstable [44] and it causes increased operating costs due to higher energy consumption of the heating units. Therefore an optimization study has been carried out to identify the highest yields achievable when the factor temperature is minimized.

3.3 Yields' optimisation

In order to predict the best factor levels that will maximize the biogas and methane production; the optimizing function consists of the maximization of eq. (4) and (5). A numerical optimisation provided by Design-Expert was applied to the RSM dataset, followed by a graphical optimization. The numerical study will provide the ideal factor levels to achieve the highest biogas and methane yields, while the graphical method investigation will result in a chart that associates the factor levels to an area of target yields defined by the user. In the numerical optimisation, levels of importance were attributed to each factor and response criteria. Factors BT and T were minimized with importance 3, while the two responses were maximized with importance 5. Factor MG was left in the same range as the RSM experiment. The optimal biogas (669 cc gTS^{-1}) and methane (292 cc gTS^{-1}) yields were identified at T=30°C after BT=11 minutes of treatment using the machine's setting MG=0 turns, allowing respectively 52% extra biogas and 51% extra methane yields when compared to untreated feedstock fermenting at the same temperature.

Finally, the graphical optimisation findings are shown in Fig. 8 at MG=0 (76 µm). The target area in yellow is delimited by two curves corresponding to the maximizing criteria set by the authors. Lower and upper limits of such areas are respectively the lowest (400 cc gTS^{-1} of biogas and 250 cc gTS^{-1} of methane) and the highest (685 cc gTS^{-1} of biogas and 430 cc gTS^{-1} of methane) yields identified by Design-Expert in the numerical optimization. Fig. 8 (A) and (B) offer a quick-approach chart to obtain operational parameters for macroalgal-based reactors in co-digestion with sludge.

4 Conclusions

The Hollander beater mechanical pretreatment is effective at enhancing both methane percentage and total biogas yield compared with untreated samples. In fact, untreated samples can produce up to an average of 332, 305 and 319 cc gTS^{-1} of biogas respectively at 50°C, 40°C and 30°C. The best results of methane conversion of treated macroalgae were achieved after 10 minutes of treatment using the minimum machine's gap (76 µm) and incubated at 50°C. Such results produced up to 651±48 cc gTS^{-1} and methane yield 425±6 cc gTS^{-1}, with about 52% biogas and 53% methane yield improvement when compared to the untreated feedstock at the same incubating temperature. An optimization study was performed with the goal of reducing the operating costs associated to the pretreatment and the incubation process at the same time. Such an optimization is aimed at minimizing the incubating temperature and the pretreatment time while maximizing the biogas yield and its methane content. The optimal biogas (669 cc gTS^{-1}) and methane (292 cc gTS^{-1}) yields were identified at T=30°C after

BT=11 minutes and MG=0 turns, allowing respectively 52% extra biogas and 51% extra methane yields when compared to untreated feedstock fermenting at the same temperature.

The Hollander beater mechanical pretreatment exhibits promising results while offering the opportunity to treat wet material without the inconvenience of drying the substrate prior to treatment. Drying is, in fact, normal practice for other mechanical treatments such as milling or grinding. With the associated extra cost that drying involves, our study is more advantageous when thinking about a large scale pretreatment. With these considerations and the promising results shown, the Hollander beater's viability should be fully investigated as a pretreatment for enhanced bioenergy production.

Acknowledgements

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

References

[1] A. Singh, S.O. Olsen, A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels, Appl. Energ. 88 (2011) 3548-3555.

[2] C. González-Fernández, B. Molinuevo-Salces, M.C. García-González, Evaluation of anaerobic codigestion of microalgal biomass and swine manure via response surface methodology, Appl. Energ. 88 (2011) 3448-3453.

[3] K.W. Jung, D.H. Kim, H.S. Shin, Continuous fermentative hydrogen and methane production from *Laminaria japonica* using a two-stage fermentation system with recycling of methane fermented effluent, Int. J. Hydrogen Energy (2012) ‘in press’ http://www.sciencedirect.com.remote.library.dcu.ie/science/article/pii/S0360319912007616.

[4] V.N. Nkemka, M. Murto, Evaluation of biogas production from seaweed in batch tests and in UASB reactors combined with the removal of heavy metals, J. Environ. Manage. 91(2010) 1573-1579.

[5] J.M. Adams, J.A. Gallagher, I.S. Donnison, Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments, J. Appl. Phycol. 21 (2009) 569-574.

[6] J.H. Park, J.J. Yoon, H.D. Park, D.J. Lim, S.H. Kim, Anaerobic digestibility of algal bioethanol residue, Bioresour. Technol. 113 (2012) 78-82.

[7] R.P. John, G.S. Anisha, K.M. Nampoothin, A. Pandey, Micro and macroalgal biomass: A renewable source for bioethanol, Bioresour. Technol. 102 (2011) 186-193.

[8] L. Ge, P. Wang, H. Mou, Study on saccharification techniques of seaweed wastes for the transformation of ethanol, Renewable Energy 36 (2011) 84-89.

[9] M.G. Borines, R.L. de Leon, M.P. McHenry, Bioethanol production from farming non-food macroalgae in Pacific island nations: Chemical constituents, bioethanol yields, and prospective species in the Philippines, Renewable Sustainable Energy Rev. 15 (2011) 4432-4435.

[10] J.Y. Lee, Y.S. Kim, B.H. Um, K. Oh, Pretreatment of Laminaria japonica for bioethanol production with extremely low acid concentration, Renewable Energy (2012) ‘in press’ http://dx.doi.org/10.1016/j.renene.2012.08.025

[11] X. Shi, K.W. Jung, D.H. Kim, Y.T. Ahn, H.S. Shin, Direct fermentation for biohydrogen production by anaerobic mixed cultures, Int. J. Hydrogen Energy 36 (2011) 5857-5864.

[12] K.W. Jung, D.H. Kim, H.W. Kim, H.S. Shin, Optimization of combined (acid + thermal) pretreatment for fermentative hydrogen production from *Laminaria japonica*

using response surface methodology (RSM), Int. J. Hydrogen Energy 36 (2011) 9626-9631.

[13] J.I. Park, J. Lee, S.J. Sim, J.H. Lee, Production of Hydrogen from Marine Macro-algae Biomass Using Anaerobic Sewage Sludge Microflora, Biotechnol. Bioprocess Eng. 14 (2009) 307-315.

[14] S. Qin, H. Lin, P. Jiang, Advances in genetic engineering of marine algae. Biotechnol. Adv. 30 (2012) 1602-1613.

[15] M. Aresta, A. Dibenedetto, G. Barberio, Utilization of macro-algae for enhanced CO_2 fixation and biofuels production: Development of a computing software for an LCA study, Fuel Abstr. Curr. Titles 86 (2005) 1679-1693.

[16] K. Gao, K.R. McKinley, Use of macroalgae for marine biomass production and CO_2 fixation: A review, J. Appl. Phycol. 6 (1994) 45-60.

[17] J.D. Murphy, K. McCarthy, The optimal production of biogas for use as transport fuel in Ireland, Renewable Energy 30 (2005) 2111-2127.

[18] V.B. Agbor , N. Cicek, R. Sparling, A. Berlin, D.B. Levin, Biomass pretreatment: Fundamentals towards application, Biotechnol. Adv. 29 (2011) 675-685.

[19] A. Gurung, S.W. van Ginkel, W.C. Kang, N.A. Qambrani, S.E. Oh, Evaluation of marine biomass as source of methane in batch test: A lab-scale study, Energy 43(2012) 396-401.

[20] A.T.W.M. Hendriks, G. Zeeman, Pretreatments to enhance the digestibility of lignocellulosic biomass, Bioresour. Technol. 100 (2009) 10-18.

[21] G.Y.S. Mtui, Recent advances in pretreatment of lignocellulosic wastes and production of value added products, Afr. J. Biotechnol. 8 (2009) 1398-1415.

[22] L. Da costa Sousa, S.P.S. Chundawat, V. Balan, B.E. Dale, ‘Cradle-to-grave’ assessment of existing lignocellulose pretreatment technologies, Curr. Opin. Biotechnol. 20 (2009) 339-347.

[23] M.J. Taherzadeh, K. Karimi, Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A ReviewInt. J. Mol. Sci. 9 (2008) 1621-1651.

[24] F. Raposo, M.A. De la Rubia, V. Fernández-Cegrí, R. Borja, Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures, Renewable Sustainable Energy Rev. 16 (2012) 861-877.

[25] N. Sarkar, S.K. Ghosh, S. Bannerjee, K. Aikat, Bioethanol production from agricultural wastes: An overview, Renewable Energy 37 (2012) 19-27.

[26] A. Mshandete, L. Bjornsson, A.K. Kivaisi, M.S.T. Rubindamayugi, B. Mattiasson, Effect of particlesize on biogas yield from sisal fibre waste, Renewable Energy 31 (2006) 2385-2392.

[27] G . Ghizzi D. Silva, M. Couturier, J.G. Berrin, A. Buléon, X. Rouau, Effects of grinding processes on enzymatic degradation of wheat straw, Bioresour. Technol. 103 (2012) 192-200.

[28] K. Izumi, Y. Okishio, N. Nagao, C. Niwa, S. Yamamoto, T. Toda, Effects of particle size on anaerobic digestion of food wasteInt. Biodeterior. Biodegrad. 64 (2010) 601-608.

[29] M. Hjorth, K. Granits, A.P.S. Adamsen, H.B. Møller, Extrusion as a pretreatment to increase biogas production, Bioresour. Technol. 102 (2011) 4989-4994.

[30] R. Chandra, H. Takeuchi, T. Hasegawa, Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production, Renewable Sustainable Energy Rev. 16 (2012) 1462-1476.

[31] L.P. Ramos, The chemistry involved in the steam treatment of lignocellulosic materials, Quím. Nova 26 (2003) 863-871.

[32] M.S. Kelly, S. Dworjanyn, The potential of marine biomass for anaerobic biogas production, Marine Estate research Centre, The Crown Estate, Scottish Association for Marine Science Oban, Argyll, Scotland, PA37 1QA, 2008.

[33] E.B. Cowling, T.K. Kirk, Properties of cellulose and lignocellulosic material substrates for enzymatic conversion processes, T. K. Biotechnology Bioengineering Symposium 6 (1976) 95-123.

[34] J.J. Cheng, G.R. Timilsina, Status and barriers of advanced biofuel technologies: A review, Renewable Energy 36 (2011) 3541-3549.

[35] S. Tedesco, M.E. Montingelli, K.Y. Benyounis, A.G. Olabi, Mechanical pretreatment effects investigation on biogas yields from different species of macroalgal biomass, Int. Conf. on Sustainable Energy & Environmental Protection (SEEP), Dublin (2012) 17-22.

[36] Verein Deutscher Ingenieure, VDI 4630, Düsseldorf, 2006.

[37] D.C. Montgomery, Design and analysis of experiments, US: John Wiley, 2005.

[38] M.E. Montingelli, S. Tedesco, M. Dassisti, A.G. Olabi, Review of mechanical and physical biomass pretreatment to increase the biogas yield, Int. Conf. on Sustainable Energy & Environmental Protection (SEEP) Dublin (2012), 2012.

[39] J.M.M. Adams, A.B. Ross, K. Anastasakis, E.M. Hodgson, J.A. Galler, J.M. Jones, I.S. Donnison, Seasonal variation in chemical composition of the bioenergy feedstock *Laminaria Digitata* for thermochemical conversion, Bioresour. Technol. 102(2011) 226-234.

[40] J.M.M. Adams, T.A. Toop, I.S. Donnison, J.A. Gallagher, Seasonal variation of *Laminaria digitata* and its impact on biochemical conversion routes to biofuels, Bioresour. Technol. 102 (2011) 9976-9984.

[41] A. Haug, A Jensen, Seasonal variations in chemical composition of *Alaria esculenta, Laminaria saccharina, Laminaria hyperborea* and *Laminaria digitata* from Northern Norway, Norw. Inst. Seaweed Res., Report No. 4, Trondheim, 1954.

[42] D.P. Chynoweth, C.E. Turick, J.M. Owens, D.E. Jerger, M.W. Peck, Biochemical methane potential of biomass and waste feedstocks, Biomass Bioenergy 5 (1993) 95–111.

[43] R. Samson, A. Leduyt, Detailed study of anaerobic digestion of *Spirulina maxima* algal biomass, Biotechnol Bioeng 28 (1986) 1014–23.

[44] D.P. Chynoweth, S. Ghosh, D.L. Klass, Anaerobic digestion of kelp, in: S.S. Sorer, O.R. Zaborsky (Eds.), Biomass Conversion Processes for Energy and Fuels, Plenum Press, New York, 1981, pp. 315-38.

Table Captions:

Table 1. Sludge characterizations used to determine the sludge contribution (Celtic Anglian Water Ltd.).

Table 2. Machine-gap and beating-time-related MC%s and TS %s.

Table 3. RSM (BBD) process variables, design levels and coded values.

Table 4. Design matrix and measured biogas and methane yields.

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Fig. 1. Hollander beater's working scheme and machine adopted in the experiment.

Fig. 2. Heating units with reactors and collection bags.

Fig. 3. Normal plot of residuals for biogas (A) and methane (B) response.

Fig. 4. Perturbation plot (A) showing the effect of process parameters on biogas volume; Interaction plot showing the effect between T and MG (B) on biogas volume.

Fig. 5. Perturbation plot (A) showing the effect of process parameters on CH_4 volume rate; Interaction plot (B) showing the effect between T and MG on CH_4 volume rate.

Fig. 6. Response surface plot showing the effect of BT and MG on biogas rate (A) and CH_4 rate (b) at T=50°C.

Fig. 7. Bar-diagram of mean biogas (A) and methane (B) measured yields; *n=2, standard deviation bars*.

Fig. 8. Optimum zone with highest software-estimated biogas (A) and CH_4 (B) yields in cc gTS^{-1} at MG=0 (76 µm).

Table 1. Sludge characterizations used to determine the sludge contribution (Celtic Anglian Water Ltd.)

Parameters	**Value**
Total Solids (TS) [%]	4.0±0.04
Volatile Solids (VS) [%]	69±1.2
COD [mg/l]	68.0±2.6
Ammonia [mg/l]	2.04±0.01
Alkalinity [mg/l]	10.6±0.40
VFA’s [mg/l]	342±22

Table 2. Machine-gap and beating-time-related MC%s and TS %s.

MG [turns*]	**BT [minutes]**	**MC [%]**	**TS [%]**
10	5	98.7	1.3
	10	98.7	1.3
	15	98.4	1.6
5	5	98.5	1.5
	10	98.6	1.4
	15	98.3	1.7
0	5	98.7	1.3
	10	98.8	1.2
	15	98.7	1.3

**0 turn=76 µm gap.*

Table 3. RSM (BBD) process variables, design levels and coded values.

Variables	**-1**	**0**	**+1**
Preatreatment time (minutes)	5	10	15
Machine chopping gap (turns)	0	5	10
Incubation temperature (°C)	30	40	50

Table 4. Design matrix and measured biogas and methane yields.

Design matrix				**Response**	
Exp. No.	**χ_1: Temperature [°C]**	**χ_2: Beating Time [minutes]**	**χ_3: Machine's Gap [turns]**	**Y_1:Volume [cc gTS^{-1}]**	**Y_2:CH_4 [cc gTS^{-1}]**
1	30	5	5	513.8	225.1
2	50	5	5	196.9	136.4
3	30	15	5	469.7	207.4
4	50	15	5	258.2	139.4
5	30	10	0	667.5	277.4
6	50	10	0	617.1	421.2
7	30	10	10	553.5	243.5
8	50	10	10	322.4	206.2
9	40	5	0	225.7	171.9
10	40	15	0	325.0	222.3
11	40	5	10	152.8	100.3
12	40	15	10	202.9	143.9
13	40	10	5	195.0	144.6
14	40	10	5	168.9	128.3
15	40	10	5	166.4	118.9
16	40	10	5	210.7	145.3
17	40	10	5	115.4	82.2
18	30	5	5	493.4	216.9
19	50	5	5	210.3	98.1
20	30	15	5	483.9	213.6
21	50	15	5	252.1	165.2
22	30	10	0	646.7	256.5
23	50	10	0	685.0	429.8
24	30	10	10	500.8	224.6
25	50	10	10	348.6	212.1
26	40	5	0	189.4	145.7
27	40	15	0	315.8	212.8
28	40	5	10	123.6	96.9
29	40	15	10	178.7	132.8
30	40	10	5	82.1	75.5
31	40	10	5	159.8	112.2
32	40	10	5	160.4	114.8
33	40	10	5	150.4	109.0
34	40	10	5	169.9	113.9

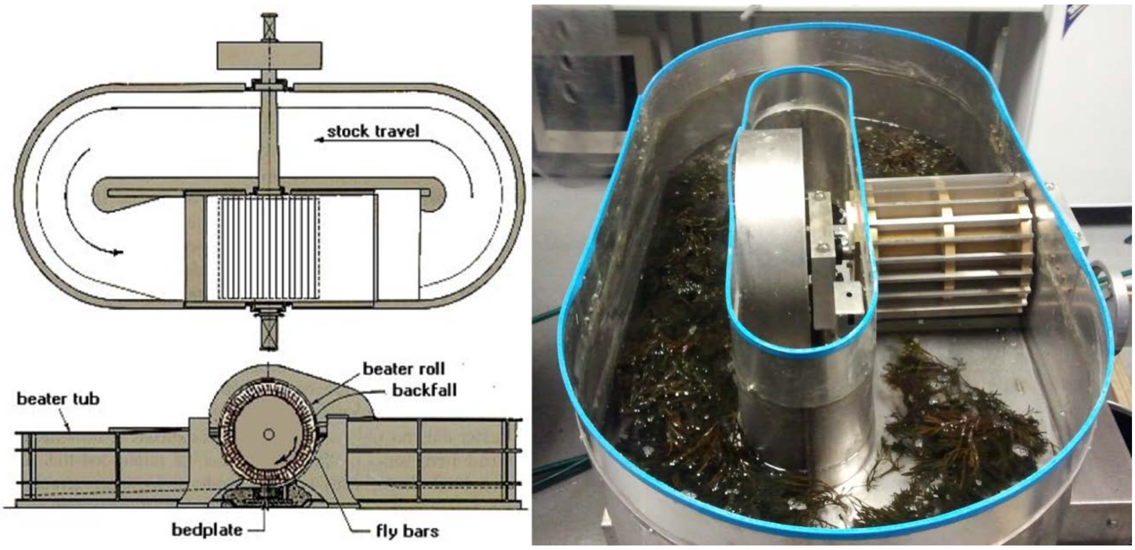

Fig. 1 Hollander beater's working scheme and machine adopted in the experiment.

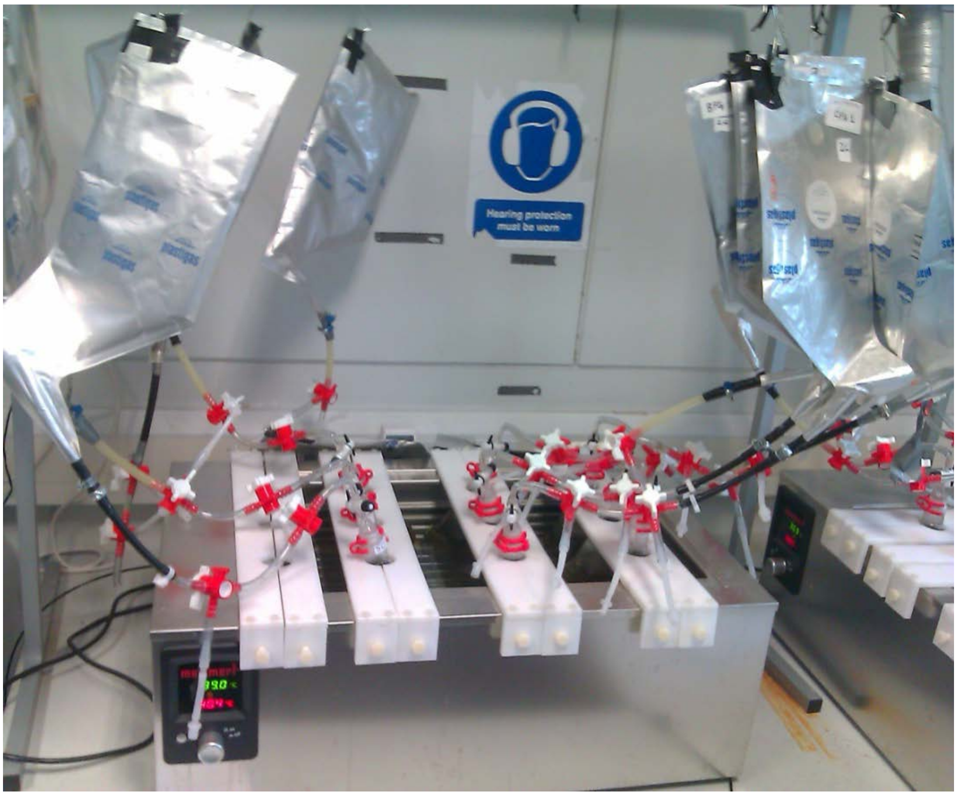

Fig. 2 Heating units with reactors and collection bags.

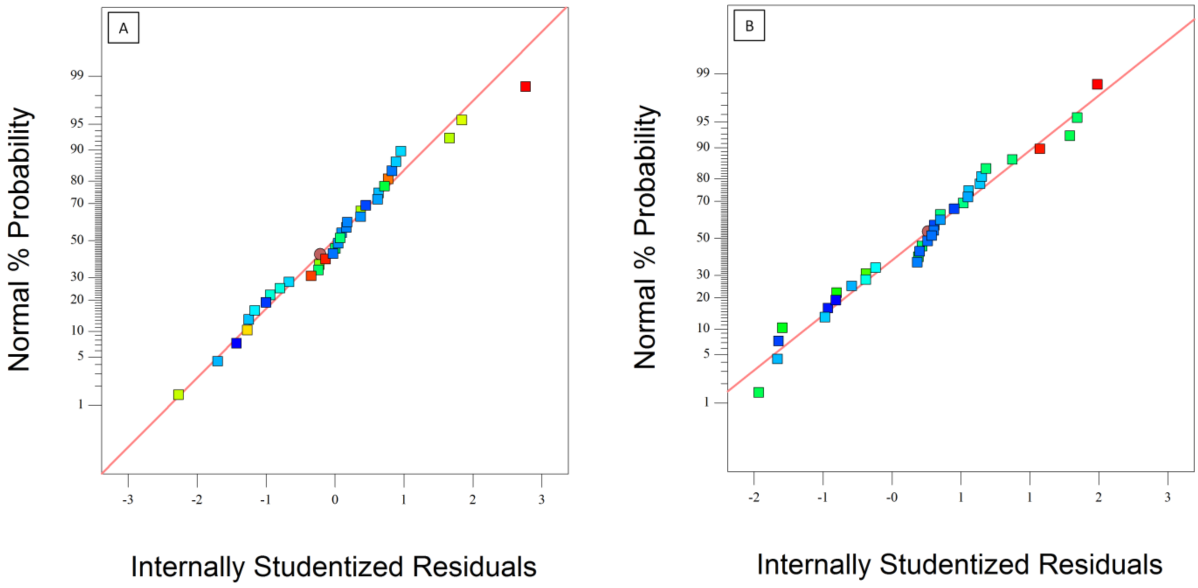

Fig. 3 Normal plots of residuals for biogas (A) and methane (B) response.

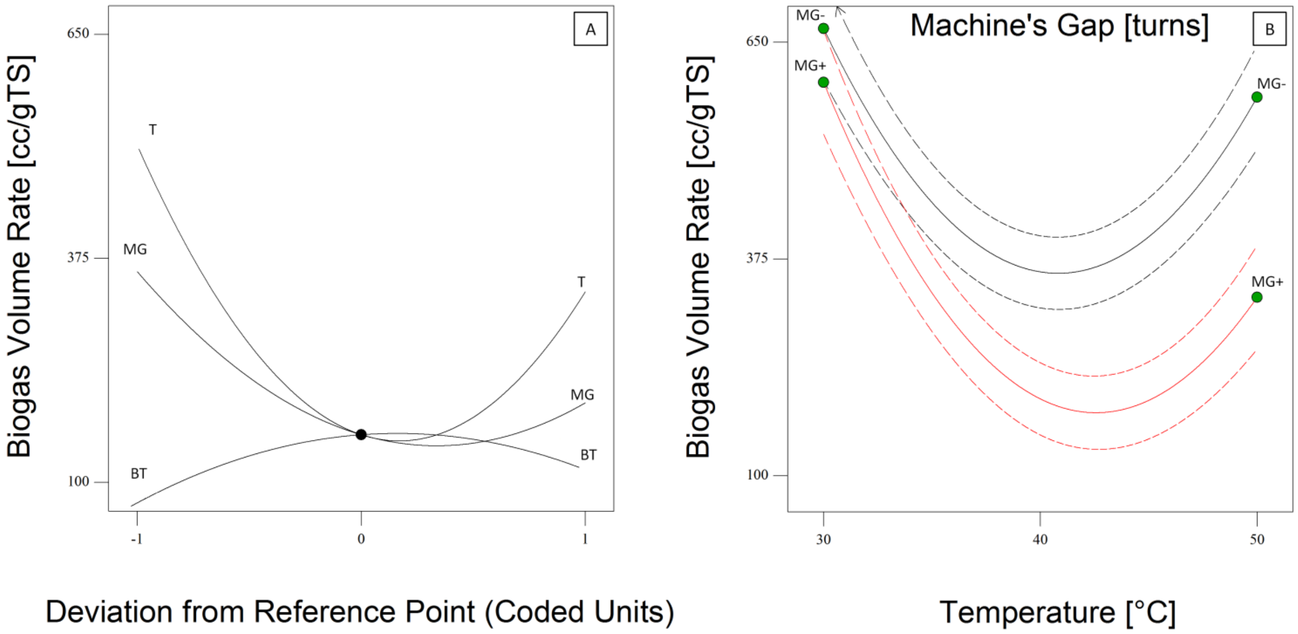

Fig. 4 Perturbation plot (A) showing the effect of process parameters on biogas volume; Interaction plot showing the effect between T and MG (B) on biogas volume.

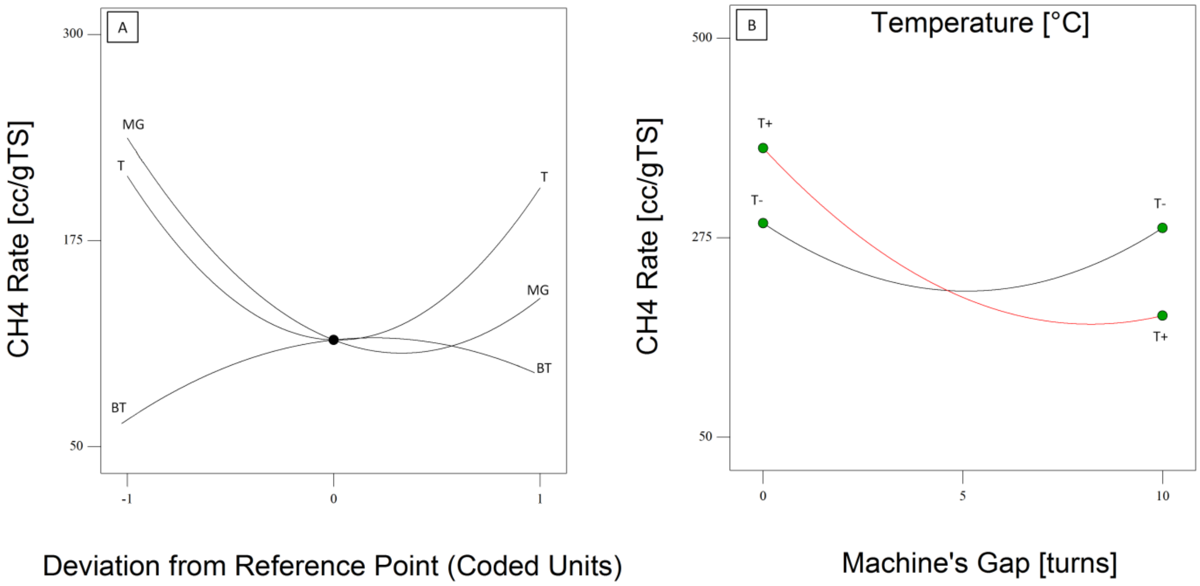

Fig. 5 Perturbation plot (A) showing the effect of process parameters on CH_4 volume rate; Interaction plot (B) showing the effect between T and MG on CH_4 volume rate.

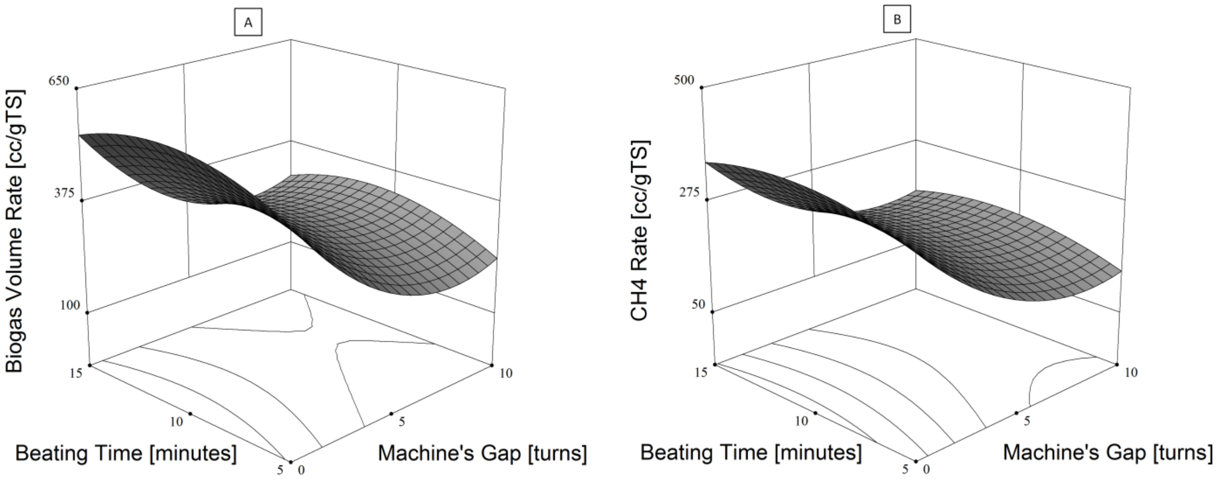

Fig. 6: Response surface plot showing the effect of BT and MG on biogas rate (A) and CH_4 rate (B) at T=50°C.

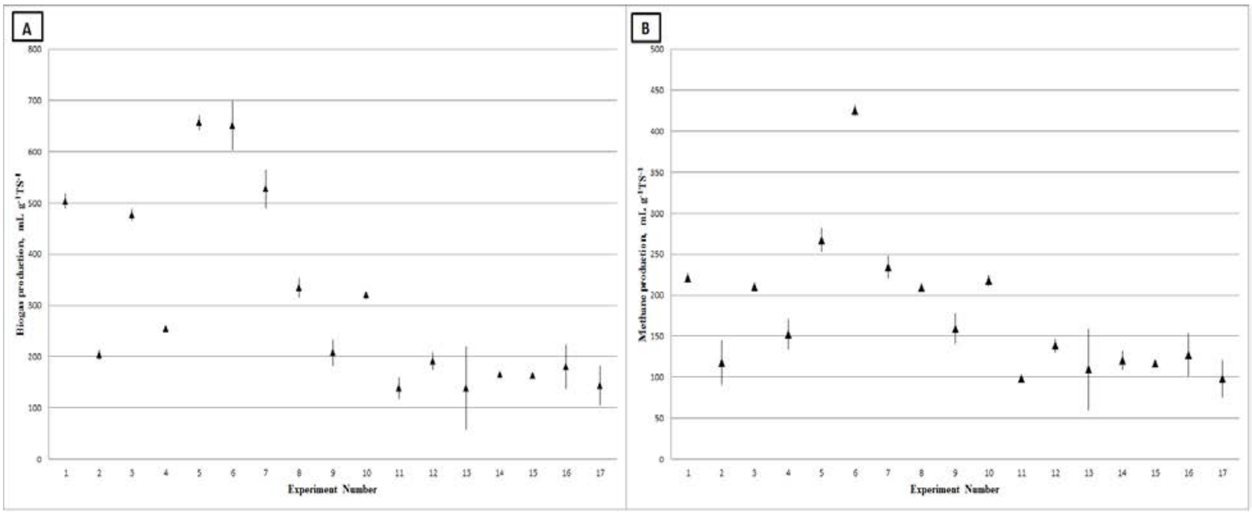

Fig. 7 Bar-diagram of mean biogas (**A**) and methane (B) measured yields; *n=2, standard deviation bars*.

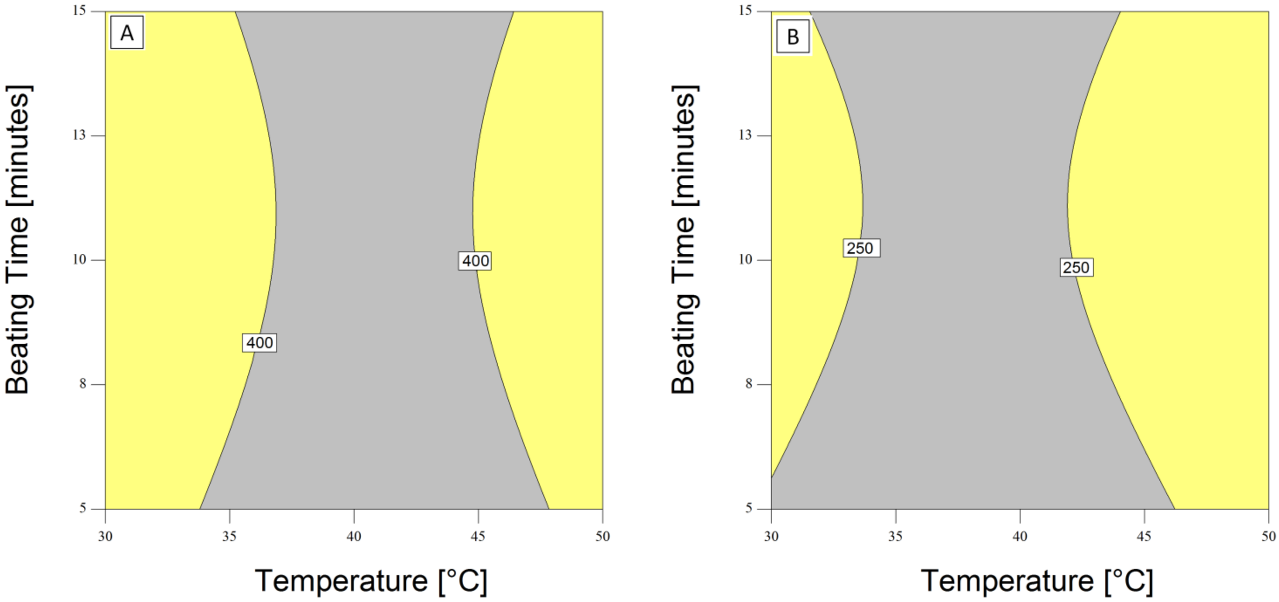

Fig. 8 Optimum zone with highest software-estimated biogas (A) and CH_4 (B) yields in cc gTS^{-1} at MG=0 (76 µm).