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**Mechanical pretreatment for increased biogas
production from lignocellulosic biomass; predicting
the methane yield from structural plant components**

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Abstract

Lignocellulosic substrates are associated with limited biodegradability due to the structural complexity. For that reason, a pretreatment step is mandatory for efficient biomass transformation which will lead to increased bioenergy output. The aim of the present study was to assess the efficiency of two pretreatment machines to enhance the methane yield of meadow grass. Specifically, the application of shearing forces with a rotated plastic sweeping brush against a steel roller significantly increased biomass biodegradability by 20% under relatively gentle operation conditions (600 rpm). The more intense operation (1200 rpm) was not associated with higher methane yield enhancement. Regarding an alternative machine, in which the brush was replaced with a coarse steel roller resulted in a more distinct effect (+27%) despite the lower rotating speed (~400 rpm). Moreover, the association of the substrate's individual chemical components and the practical methane yield was assessed, establishing single and multiple linear regression models. However, the estimation accuracy was rather low with either single (regressor: lignin, R^2 : 0.50) or multiple linear regression analyses (regressors: arabinan-lignin-protein, R^2 : 0.61). Results showed that poorly lignified plant tissue containing relatively high fractions of protein and arabinan is more susceptible to anaerobic digestion.

Keywords

Biogas; Mechanical pretreatment; Methane yield; BMP prediction model; Principal component analysis

1. Introduction

Agricultural land (i.e. arable land, permanent crops, permanent meadows and pastures) covers more than 37% of the total land surface with lignocellulosic biomass as the main organic component (Faostat, 2016). Lignocellulosic biomass is considered as a potential feedstock for industrial biofuels production (e.g. biogas, bioethanol). This application has several advantages, e.g. besides of being CO₂ neutral, it is contributing to lower emissions compared to the common practice of burning the lignocellulosic residues on the field (Hayashi et al., 2014; Marañón et al., 2011). Among the different technologies for biofuel production using lignocellulosic materials, anaerobic digestion appears as the most promising application as it is more energetically favourable compared to liquid biofuels (Frigon and Guiot, 2010).

The biomass' characteristics, such as plant size, shape and moisture content are crucial for efficient biomethanation and also, optimal process performance. Thus, lignocellulosic materials need to be pretreated prior to their introduction in the anaerobic reactor in order to avoid operational problems (e.g. clogging, floating layers) (Mönch-Tegeder et al., 2014). Moreover, the pretreatment is essential in order to minimise the resistance of the solid feedstocks to enzymatic attack. Indeed, it is mandatory to disrupt the complex lignocellulosic structure in order to improve the conversion of structural carbohydrates to monomeric sugars (Katukuri et al., 2017; Ranjan and Moholkar, 2013). On this topic, it was recently shown that mechanical comminution machines can efficiently improve the biomass characteristics for biogas applications (i.e. size reduction, surface disruption, partial drying) (Tsapekos et al., 2017a). In general, mechanical pretreatments are considered more appropriate for industrial applications compared to the alternative methods (i.e. thermal, chemical,

biological) (Carrere et al., 2015). The mode of action of mechanical methods relies on the application of shear and/or compression forces to boost biomass deconstruction (Kratky and Jirout, 2011). With respect to operational features, the selection of suitable construction materials is of major importance. Indeed, it was reported that using materials of high coarseness to equip a comminution device, the biogas enhancement could be significantly improved compared to other smoother materials (Tsapekos et al., 2015).

Moreover, for the overall sustainability of the pretreatment method, durability and robustness of equipment is of high importance. For instance, the performance of equipment can be deteriorated in real-life applications and in cases that large materials other than biomass are processed in error (e.g. metal pieces or stones). Thus, machine's lifetime can be markedly decreased or the equipment can collapse adding extra costs. In this context, the controlled and optimized operation of full-scale harvesting machines can remarkably reduce the energy input and in parallel, increase the output of lignocellulose-based AD (Tsapekos et al., 2017). Likewise, it was revealed that the energy balance of AD using various crops is significantly affected by the performance of the used comminution machine (Herrmann et al., 2012). Hence, both a careful design of equipment in terms of materials and technology and also, the proper usage should always be ensured in order to efficiently apply vigorous mechanical forces and consequently, increase substrate's biodegradability.

Of equal importance with pretreatment's efficiency for high bioenergy output are the feedstock's characteristics. For instance, biomass biodegradability is negatively correlated to the level of cell walls lignification (Wang et al., 2009). In contrast, ensiled substrates are possibly associated with high methane production due to the fermentation

of complex carbohydrates into metabolic intermediates (e.g. lactic acid) that took place during the ensiling process (Vervaeren et al., 2010). Despite well-established knowledge about the relations of biomass characteristics and biodegradability, a standardised and constant correlation between chemical characteristics and biochemical methane potential (BMP) is not yet existing (Dandikas et al., 2015). In addition, the BMP measurement provides important information about substrate's biodegradability but it is a time-demanding process (Charnier et al., 2016). Conversely, the analytical methods for biomass characterization are only requiring relatively short time. Hence, linking prompt methods with the BMP values could establish a fast decision-making tool in order to design and apply strategies that can lead to process optimisation in full-scale applications (Fitamo et al., 2017). In a recent study, more than 200 samples were used to correlate the hydrolysis rate constant with the chemical composition of different biomasses, as a precursor for predicting the biogas production (Dandikas et al., 2018). The non-fibre structural sugars and crude protein contents were associated with the most efficient, but still moderate, prediction accuracy ($R^2 \sim 66\%$). Thus, the suggested approach can be followed for an initial assessment of feedstock degradation potential; however, a strong correlation that leads to accurate forecasting cannot be expected. Similarly, Edwiges et al. (2018) examined the correlation between the compositional components of fruit-vegetable residues and methane potential. Results interpretation revealed that lignin content is a strong predictor of solid waste, which is in accordance with a similar previous study (Triolo et al., 2011). However, lignin is significantly higher at agricultural residues compared to the fruit-vegetable waste, and additionally, its chemical structure differs markedly among the various lignocellulosic species. Moreover, lignocellulose characteristics, such as crystallinity, degree of polymerization,

content and composition of monolignol precursors, can indirectly affect the anaerobic biodegradability. Hence, linking the chemical synthesis with the practical methane yield is still challenging.

The present study aims to elucidate the efficiency of two mechanical pretreatment methods for enhancing the biogas production from meadow grass. The performance was evaluated with respect to different operational parameters (i.e. different rotating speeds and biomass loads). Meadow grass was used a model biomass for the pretreatment tests due to its high availability in marginal lands and environmentally sensitive areas. Specifically, the meadow grass that is not used for grazing during summer due to its low fodder value can with great benefit be exploited for bioenergy purposes. Moreover, the correlation among substrate's chemical characteristics and methane yield was examined to develop a rapid and reliable model to predict lignocellulose's biodegradability. The development of an efficient prediction tool could allow the biogas plant operators to successfully assess the energy output that can be obtained by a given substrate. This application signifies, its high importance for real-life practices. On this subject, a heterogeneous dataset using various agricultural substrates were used to develop a prediction model.

2. Materials and methods

2.1. Inoculum

Inoculum was obtained from the effluent tank of a thermophilic biogas plant (Snertinge, Denmark). The inoculum was immediately transported to the laboratory and subsequently, was sieved to remove particles larger than 10 mm. Afterwards, the inoculum was incubated for 10 days at thermophilic temperature to remove background

methane production. The main chemical characteristics of the inoculum were: pH: 8.07, Total Solids (TS): 24.1 ± 0.1 g/L, Volatile Solids (VS): 15.0 ± 0.1 g/L, Total Kjeldahl Nitrogen (TKN): 4.6 ± 0.2 g-TKN/L, Ammonium Nitrogen: 3.8 ± 0.1 g NH_4^+ -N/L, and total Volatile Fatty Acids (TVFA): 0.7 ± 0.1 g/L.

2.2. Substrates

To define the correlation between methane production and lignocellulosic substrates' chemical composition, diverse species were examined; grass obtained from various meadows (i.e. M1-M7), grass clippings (GC), wheat straw (WS) and digested biofibers (DB). Samples of non-cultivated meadow grass were manually collected from natural grasslands. The meadow fields were mainly consisted of white clover, tetraploid hybrid ryegrass, diploid early perennial ryegrass and diploid medium-early perennial ryegrass. For proper silage fermentation, the grasses were kept intact at room temperature for two days to be partially dried, and then, were placed in anaerobically sealed plastic bags without injecting biological additives for sixty days. Grass clippings and wheat straw were collected from fields in Zealand, Denmark. Overall, ten different lignocellulosic substrates were examined in triplicate samples. The chemical composition of the used substrates is presented in Table 1. Samples were ground to 2 mm to determine their chemical characteristics.

2.3. Mechanical pretreatment

Meadow grass was used in order to assess the efficiency of mechanical pretreatments on biogas enhancement (Table 2). Two different mechanical pretreatment units were constructed in order to evaluate their effect on the anaerobic biodegradability

of meadow grass. The first machine was consisted of a road sweeping brush and a steel mesh conditioner (crimper) (Fig. 1a). Pretreatment's efficiency was based on the principle that the plastic brush roller, which was placed diametrically opposed to the steel roller can intensively press the biomass towards the reversely rotating equipment. The brush roller was rotated at different driving speeds in order to define the most optimal operation (Table 2). Conversely, the steel roller was always rotating in the same speed as its main usage was to ensure a proper grass flow towards the brush roller. During all trials, the same amount of grass (1.4 kg/m^2) was treated to simulate the application of a full-scale harvester at a typical meadow field. Regarding the second test, the comminuting apparatus was partially amended in order to enhance the damage of biomass surface. Hence, a corrugated roller with coarse steel surface was rotating towards a stable coarse shell (Fig. 1b). Additionally, different amounts of grass were pretreated in order to operate the unit at different biomass loads. During both tests, the grass was placed on a conveyor belt, which supplied the biomass to the pretreatment units. In order to simulate the procedure in full-scale, the conveyor was operated at the same driving speed of a mower at a real field (10 km/h).

2.4. Batch experiments

Batch assays were conducted to determine the Biochemical Methane Potential (BMP) of untreated and pretreated substrates according to the guidelines described by (Angelidaki et al., 2009). Glass bottles of 547 mL total volume were employed and filled with specific amounts of water, inoculum and substrate to reach working volume of 200 mL and initial organic load of 2 gVS/L. The inoculum consisted 80% (v/v) of the working volume to ensure that the volume of microbial biomass was sufficient enough

to efficiently digest the organic matter. Thus, the batch assays were set-up at inoculum to substrate ratio equal to 8. The adjustment of the initial organic load targeted given value was performed by supplementing the batch vials with water. The inoculum content was always kept constant. The reactors were flushed with 100% N₂ to ensure anaerobic conditions and subsequently, placed in thermophilic incubator (54 ± 1 °C). Manual mixing of the batch reactors and gas measurements were conducted in a regular basis until cease of methane production ($p < 0.05$).

2.5. Analytical methods

Measurements of TS, VS, pH, NH₄⁺-N and protein were performed according to the standard methods for the examination of water and wastewater (APHA, 2005).

Structural carbohydrates (i.e. glucan, xylan, arabinan) and Klason lignin were measured according to (Sluiter et al., 2011). The quantification of carbohydrates was performed using Ion chromatography (Dionex ICS-5000, Thermo Scientific). An analytical column (CarboPak PA1, 250 × 4 mm) was employed for sugars separation using 100% Milli-Q water as first eluent, 1M NaOH as second eluent and 100mM NaOH as third eluent; at a constant flow rate of 1.0 mL/min. Gas chromatography (Shimadzu GC-8A, Japan) was used to determine the BMP of the lignocellulosic substrates and the volatile fatty acids (VFA) composition of the inoculum (Kougias et al., 2015). The carbon to nitrogen ratio (C:N) of lignocellulosic substrates was defined using a vario MACRO cube, CHNOS elemental analyzer (Hanau, Germany). All the physicochemical analyses were conducted in triplicate samples. Scanning Electron Microscopy (SEM) images were taken using a FEI Quanta 200 F equipped with a Field Emission Gun (FEG) to illustrate the damages on the grass morphology provoked by the comminution machines. Freeze

dried lignocellulosic biomasses were placed onto aluminium SEM specimen stubs with carbon conductive tabs and coated with gold for higher conductivity.

2.6. Data analysis

The effect of mechanical pretreatments on the kinetics of grass anaerobic degradation was evaluated using the modified Gompertz equation:

$$M(t) = M_0 \times \exp \left\{ -\exp \left[\frac{R_{max} \times e}{M_0} (\lambda - t) + 1 \right] \right\}$$

where:

$M(t)$ = accumulated CH₄ yield at digestion time t (mLCH₄/gVS),

M_0 = CH₄ yield (mL/gVS),

R_{max} = maximum CH₄ production rate (mL/gVS/d),

λ = lag phase (day),

e = Euler's constant (2.7183).

The coefficient of determination (R^2) and the root mean square error (RMSE) were calculated in order to evaluate the prediction accuracy of the models (Tsapekos et al., 2015). Tukey post hoc test ($p < 0.05$) and one-way analysis of variance (ANOVA) was followed to define any statistically significant differences among untreated and pretreated samples using the software Graphpad Prism (Graphpad Software, Inc., San Diego, CA). The Kolmogorov–Smirnov test was applied ($p < 0.05$) to determine the normal distribution of the samples. Descriptive statistics and principal component analysis (PCA) were performed to examine the correlations among chemical characteristics. Moreover, multiple linear regression analysis was conducted in order to predict the methane yield of lignocellulosic substrates. To define the prediction accuracy, the coefficient of variation (CV) and normalised root mean square error

(NRMSE) were also calculated. The software XLSTAT (Addinsoft, New York, NY) was used for the analysis.

3. Results and discussion

3.1. BMP experiments

The methane yields of untreated and pretreated grass with both pretreatment units are summarized in Table 2. Regarding the first pretreatment unit, the most profound enhancement (20%, $p < 0.05$) was achieved at the moderate rotating conditions (600 rpm) (Fig. 2a). Apart from the most optimal operation, no significant differences ($p > 0.05$) were observed for the rest trials in comparison with untreated operation. Moreover, the highest rotating speeds (i.e. 900 and 1200 rpm) had similar effect ($p > 0.05$) on biomethanation compared to the smoothest operation (i.e. 200 rpm). This result is contradictory with a previous study in which grass biodegradability was proportionally related to the increased harvesting speed of full-scale machines (Tsapekos et al., 2017). The efficiency of pretreatment units is not always directly correlated with the rotational speed. It is mainly the disruption technology that highly influences the level of plant's structural disruption (Kratky and Jirout, 2011) and at a lesser extent, the rotational speed. In this context, Boscaro et al. (2015) suggested that flail or disc mowing are appropriate solutions for extracting grass from the fields to supply AD plants. Flail or disc mowers are based on rotating horizontal drums which are similar to the constructed pretreatment unit.

In the present study, the grass flow through the rollers could be improved at moderate rotating speed avoiding blockage of the equipment and thus, the demanding shearing forces were more efficiently applied. Apart from the increments on methane

yield, the kinetic parameters were also influenced during pretreatment trials (Table 2). Specifically, the examined methods insignificantly ($p > 0.05$) decreased the lag phase of pretreated samples according to the modified Gompertz model. The results are in accordance with a previous research, in which the performance of full-scale machines on mechanical disruption was evaluated demonstrating that the utilisation of the most efficient machinery led to a reduction of digestion's lag phase (Tsapekos et al., 2017a). Actually, during the AD of lignocellulosic biomass, lag phase is typically detected due to high structural complexity of these substrates. Hence, a model that takes into account this acclimation period is considered to be appropriate for parameter's estimation (Kafle and Chen, 2016). In this context, the good fit of the model was observed by the high R^2 and low RMSE values and thus, reliable prediction accuracy was concluded (Raju et al., 2011). Moreover, the methane production rates were only slightly improved ($p > 0.05$) in the majority of the pretreated samples.

As a continuation of the first AD trial, a consecutive BMP test was conducted in order to examine the effect of a modified version of the machine on the biodegradability of lignocellulosic biomass. Specifically, the plastic brush roller was replaced by a coarse corrugated roller. The modifications were conducted based on previous findings which demonstrated that lignocellulosic structural changes are intensified with the application of shearing forces using mesh gratings of coarse steel (Tsapekos et al., 2015). Additionally, the pretreatment unit was always operated at one rotating speed (~ 400 rpm) as the first BMP test showed that the enhancement of anaerobic biodegradability is not directly connected with roller's rotating speed. The results from AD trials showed that the modified pretreatment machine improved the process performance compared to the initial model, as the biogas production was significantly enhanced ($p < 0.05$) in the

range of 22-27% (Table 2; Fig. 2b). Moreover, it was found that the variation in the biomass loadings did not have any significant effect ($p > 0.05$) on the biodegradability (e.g. increase of CH₄ yield, decrease of lag phase). Interesting results were obtained regarding the differences on methane production rate. More specifically, at the lowest (i.e. T2_a) and highest (i.e. T2_e) biomass loads the R_{max} values were slightly lower compared to the untreated samples. Indeed, the positive effect of T2_a and T2_e on methanation was detected after 12 and 10 days of incubation, respectively. On the contrary, T2_b-T2_d showed an immediate positive effect on methanation since the first days of AD (Table 2; Fig. 2b). Overall, a direct correlation between biomass load and kinetic parameters cannot be drawn.

These observations are particularly important as at real grass fields the biomass yield does not have a constant value but considerably varies due to heterogeneity of growth stages, species etc. (Kreuger et al., 2011; Molinuevo-Salces et al., 2013). Hence, intense vegetation is detected at meadows and marginal lands that can markedly eliminate the cutting capacity of harvesters. Interestingly, the obtained results revealed that pretreatment's efficiency was not deteriorated by increasing the amount of loaded biomass. Thus, similar levels of efficiency could be achieved under non-controlled conditions (i.e. real-life applications) and not only under optimized conditions (i.e. lab-scale tests) in which limited amount of biomass is processed per trial. In addition, the ability of pretreatment units to create structural damages on the biomass morphology was revealed by the SEM analysis (Fig. 3). In this context, the efficiency of plastic brush roller to peel the grass surface and increase the biodegradable area for the microbial attack was revealed (Fig. 3b). Regarding the effect of the modified unit on the structural changes, an increased number of small grass particles were observed due to

the usage of coarse metal materials (Fig. 3c).

Focusing on full-scale applications, harvester equipped with similar apparatus that apply shearing forces managed to pretreat efficiently lignocellulosic biomass and consequently, could improve the methane yield up to 20% (Tsapekos et al., 2017a). Although different levels of methane yield enhancement by mechanical pretreatment methods have been reported (from 8.5 to 62%); it can be noticed that in general, the impact of applied pretreatments have positive impact on substrate's biodegradability (Rodriguez et al., 2016). In addition, the suitability of full-scale comminution machines on improving the energy budget of grass-based AD was previously proved (Tsapekos et al., 2017b). In the mentioned study, the produced biogas (output) was compared with machine's energy consumption (input) and the analysis showed that a highly efficient full-scale machine is able to improve the overall sustainability of grass based-AD. The mechanical pretreatment methods pose the characteristics for environmentally friendly management implications of waste and residues. To sum up, both applied pretreatment units could induce structure damage, by presumably increasing surface area and thus, improving the access of AD microorganisms to the degradable organic matter (Tedesco et al., 2014).

3.2. Correlation between BMP and chemical composition

Results obtained from the chemical characterization of the examined lignocellulosic substrates and their corresponding BMPs are summarized in Table 1. The BMP values were normally distributed and represented a wide range in methane yields (i.e. from 34 to 524 mL/gVS). In fact, datasets with wide range of values are appropriate for linking the chemical components and BMP values of lignocellulosic substrates (Dandikas et al.,

2018; Raju et al., 2011). Regarding the examined variables, only C:N ratios were not normally distributed and thus, were not suitable parameters for mathematical regression analyses.

Initially, PCA was applied to datasets (i.e. content of glucan, xylan, arabinan, lignin and protein) in order to reveal the correlations among the variables. The PCA biplot shows that the first two principal components explained 91% of total variation (Fig. 4). Taking into account the contents of lignin, xylan, glucan, arabinan and protein, the variations explained by PC1 (reflecting the linear combination of x-variables that has the highest variance) and PC2 (reflecting the linear combination of x-variables accounting for the potential highest remaining variations) were 49% and 42%, respectively. All variables defined to be away from centre and close to the surrounding circular area, indicating that samples variation was considerably explained (Fig. 4). Hence, they were used to investigate possible correlations between the parameters. Among variables, lignin and protein were highly inversely correlated ($r=-0.87$, angle close to 180°) and on the other hand, lignin and glucan were highly independent ($r = 0.04$, angle $\sim 90^\circ$) (Table 3; Fig. 4). Regarding the link between chemical components and methane yield, lignin polymer revealed the closest relationship ($r = -0.71$, $p > 0.0001$) (Table 1). The observed negative correlation is in accordance with previous studies that used lignin as regressor to predict the biodegradability of lignocellulosic substrates (Campuzano and Gonzalez-Martinez, 2016; Dandikas et al., 2014; Edwiges et al., 2018; Herrmann et al., 2016). Single linear regression was established using lignin but the calibration statistics were relatively low ($R^2 = 0.50$, RMSE = 75 mL/gVS, NRMSE = 15%). The model is described by equation 1:

$$CH_4 \text{ yield} = 512.44 - 6.69 \times \text{Lignin} \quad (1)$$

where, CH_4 *yield* is given in mL/gVS and lignin in g/100gVS.

Previous studies concluded that simple linear regression analysis using the chemical characteristics of substrates cannot lead in high estimation accuracy, but can be used as an initial and time-effective prediction approach (Dandikas et al., 2014; Edwiges et al., 2018; Lesteur et al., 2010). However, the prediction accuracy could be improved by applying multiple linear regression models (Kafle and Chen, 2016). Hence, a multiple regression test was performed using the contents of arabinan, lignin and protein as regressors and CH_4 yield as regressand. According to the PCA analysis, glucan and xylan units were insignificant for the model ($p < 0.05$) and thus, were not further used. Furthermore, outliers were not detected, as the square root of the ratio using the residual value per observation and the average residual variance was always lower than 3.0 (Dandikas et al., 2015). The analysis showed that the prediction accuracy was improved, as the R^2 of the model was 0.61, the RMSE was 68 mL/gVS and the NRMSE was 14%. The Kolmogorov–Smirnov test was applied to examine the normal distribution of the residuals and the null hypothesis could not be rejected. The model is described by equation 2:

$$\text{CH}_4 \text{ yield} = 270.74 + 16.61 \times \text{Arabinan} - 3.35 \times \text{Lignin} + 27.88 \times \text{Proteins} \quad (2)$$

where, CH_4 *yield* is given in mL/gVS and the chemical compounds in g/100gVS.

Linking the chemical composition of lignocellulosic biomass and BMP values was previously assessed using similar variables (Dandikas et al., 2015). Specifically, the usage of protein, lignin and hemicellulose as independent variables resulted in multiple linear regression model with a relatively good accuracy ($R^2 = 0.70$). The better prediction accuracy compared to the present study can be attributed to the large number of grass species that were harvested at different growth stages. Therefore, better

estimation accuracy was achieved due to the higher variation in significant parameters (e.g. lignin content). In another study the researchers used similar regressors (i.e. lignin and protein) in addition to two alternative components (i.e. butyric acid and alcohols), achieving prediction accuracy ($R^2 = 0.64$, RMSE = 27 mL/gVS) comparable to the present work (Herrmann et al., 2016). The slightly improved calibration statistics, obtained in the study of (Herrmann et al., 2016), can be ascribed to the usage of a huge dataset containing various silages (405 samples of 35 crop species). Hence, a very heterogeneous dataset with a broad range of values was utilized that positively affects the prediction accuracy (Lesteur et al., 2010). Apart from the number of samples, the type of the lignocellulosic materials used in previous and current study differs also markedly. Specifically, in the work of Herrmann et al. only silages were used and thus, the silage fermentation products were used as regressors (Herrmann et al., 2016). In contrast, the current research examined diverse lignocellulosic samples (i.e. silage, fresh, straw and digested) containing a wider variety of chemical components. Thus, the silage fermentation products would have extremely limited applicability in our study as the majority of examined lignocellulosic samples contain limited or negligible concentration of these intermediates. Overall, the chemical composition of a substrate can give important information regarding its optimal methane potential and additionally, this information can be used as early indicators for substrate's biodegradability. However, parameters that were not considered in this study (e.g. crystallinity, polymerization degree, content of monolignols p-coumaryl, coniferyl and sinapyl alcohols) can be also responsible for the low prediction accuracy. Nevertheless, findings from the present case study and literature conclude that the chemical synthesis of lignocellulosic substrates can give relatively moderate estimation of anaerobic

biodegradation. Hence, it is still extremely difficult to find efficient and alternative methods to rapidly define the ultimate biodegradability of complex materials.

4. Conclusions

The application of two mechanical pretreatment units revealed to affect positively the methane yield and lag phase of grass-based anaerobic digestion process. The most aggressive machine enhanced the methane yield of meadow grass up to 27% compared to untreated samples. Moreover, the performance of the amended pretreatment unit was not negatively affected by treating increased biomass loads. Single and multiple linear regressions models were built to predict the methane production of diverse lignocellulosic substrates. Moderate correlation was noted ($R^2 = 0.61$) using arabinan, lignin and protein as independent variables. The increased complexity of lignocellulosic polymer is the major impediment to achieve high prediction accuracy using mathematical regression analyses.

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References

- Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science & Technology* 59, 927.
- APHA, 2005. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC, USA.
- Boscaro, D., Pezzuolo, A., Grigolato, S., Cavalli, R., Marinello, F., Sartori, L., 2015.

421 Preliminary analysis on mowing and harvesting grass along riverbanks for the
 422 supply of anaerobic digestion plants in north-eastern Italy. *Journal of Agricultural*
 423 *Engineering* 46, 100–104.

424 Campuzano, R., Gonzalez-Martinez, S., 2016. Characteristics of the organic fraction of
 425 municipal solid waste and methane production: A review. *Waste Management* 54,
 426 3–12.

427 Carrere, H., Antonopoulou, G., Affes, R., Passos, F., Battimelli, A., Lyberatos, G.,
 428 Ferrer, I., 2015. Review of feedstock pretreatment strategies for improved
 429 anaerobic digestion: From lab-scale research to full-scale application. *Bioresource*
 430 *Technology* 199, 386–397.

431 Charnier, C., Latrille, E., Jimenez, J., Lemoine, M., Boulet, J.-C., Miroux, J., Steyer, J.-
 432 P., 2016. Fast characterization of solid organic waste content with near infrared
 433 spectroscopy in anaerobic digestion. *Waste Management* 59, 140–148.

434 Dandikas, V., Heuwinkel, H., Lichti, F., Drewes, J.E., Koch, K., 2014. Correlation
 435 between biogas yield and chemical composition of energy crops. *Bioresource*
 436 *technology* 174, 316–20.

437 Dandikas, V., Heuwinkel, H., Lichti, F., Drewes, J.E., Koch, K., 2015. Correlation
 438 between biogas yield and chemical composition of Grassland Plant Species.
 439 *Energy & Fuels* 29, 7221–7229.

440 Dandikas, V., Heuwinkel, H., Lichti, F., Eckl, T., Drewes, J.E., Koch, K., 2018.
 441 Correlation between hydrolysis rate constant and chemical composition of energy
 442 crops. *Renewable Energy* 118, 34–42.

443 Edwiges, T., Frare, L., Mayer, B., Lins, L., Mi Triolo, J., Flotats, X., de Mendonça
 444 Costa, M.S.S., 2018. Influence of chemical composition on biochemical methane
 445 potential of fruit and vegetable waste. *Waste Management* 71, 618–625.

446 Faostat, 2016. Food and Agriculture Organization of the United Nations - Statistical
 447 Databases [WWW Document]. URL <http://faostat.fao.org> (accessed 5.1.16).

448 Fitamo, T., Triolo, J.M., Boldrin, A., Scheutz, C., 2017. Rapid biochemical methane
 449 potential prediction of urban organic waste with near-infrared reflectance
 450 spectroscopy. *Water Research* 119, 242–251.

451 Frigon, J.C., Guiot, S.R., 2010. Biomethane production from starch and lignocellulosic
 452 crops: A comparative review. *Biofuels, Bioproducts and Biorefining* 4, 447–458.

453 Hayashi, K., Ono, K., Kajiura, M., Sudo, S., Yonemura, S., Fushimi, A., Saitoh, K.,
 454 Fujitani, Y., Tanabe, K., 2014. Trace gas and particle emissions from open burning
 455 of three cereal crop residues: Increase in residue moistness enhances emissions of
 456 carbon monoxide, methane, and particulate organic carbon. *Atmospheric*
 457 *Environment* 95, 36–44.

458 Herrmann, C., Idler, C., Heiermann, M., 2016. Biogas crops grown in energy crop
 459 rotations: Linking chemical composition and methane production characteristics.

460 Bioresource Technology 206, 23–35.

461 Herrmann, C., Prochnow, A., Heiermann, M., Idler, C., 2012. Particle Size Reduction
462 During Harvesting of Crop Feedstock for Biogas Production II: Effects on Energy
463 Balance, Greenhouse Gas Emissions and Profitability. *Bioenergy Research* 5, 937–
464 948.

465 Kafle, G.K., Chen, L., 2016. Comparison on batch anaerobic digestion of five different
466 livestock manures and prediction of biochemical methane potential (BMP) using
467 different statistical models. *Waste Management* 48, 492–502.

468 Katukuri, N.R., Fu, S., He, S., Xu, X., Yuan, X., Yang, Z., Guo, R.-B., 2017. Enhanced
469 methane production of *Miscanthus floridulus* by hydrogen peroxide pretreatment.
470 *Fuel* 199, 562–566.

471 Kougiass, P.G., Boe, K., Einarsdottir, E.S., Angelidaki, I., 2015. Counteracting foaming
472 caused by lipids or proteins in biogas reactors using rapeseed oil or oleic acid as
473 antifoaming agents. *Water Research* 79, 119–127.

474 Kratky, L., Jirout, T., 2011. Biomass Size Reduction Machines for Enhancing Biogas
475 Production. *Chemical Engineering & Technology* 34, 391–399.

476 Kreuger, E., Prade, T., Escobar, F., Svensson, S.-E., Englund, J.-E., Björnsson, L.,
477 2011. Anaerobic digestion of industrial hemp—Effect of harvest time on methane
478 energy yield per hectare. *Biomass and Bioenergy* 35, 893–900.

479 Lesteur, M., Bellon-Maurel, V., Gonzalez, C., Latrille, E., Roger, J.M., Junqua, G.,
480 Steyer, J.P., 2010. Alternative methods for determining anaerobic biodegradability:
481 A review. *Process Biochemistry* 45, 431–440.

482 Marañón, E., Salter, A.M., Castrillón, L., Heaven, S., Fernández-Nava, Y., 2011.
483 Reducing the environmental impact of methane emissions from dairy farms by
484 anaerobic digestion of cattle waste. *Waste Management* 31, 1745–1751.

485 Molinuevo-Salces, B., Larsen, S.U., Ahring, B.K., Uellendahl, H., 2013. Biogas
486 production from catch crops: Evaluation of biomass yield and methane potential of
487 catch crops in organic crop rotations. *Biomass and Bioenergy* 59, 285–292.

488 Mönch-Tegeder, M., Lemmer, A., Oechsner, H., 2014. Enhancement of methane
489 production with horse manure supplement and pretreatment in a full-scale biogas
490 process. *Energy* 73, 523–530.

491 Raju, C.S., Ward, A.J., Nielsen, L., Møller, H.B., 2011. Comparison of near infra-red
492 spectroscopy, neutral detergent fibre assay and in-vitro organic matter digestibility
493 assay for rapid determination of the biochemical methane potential of meadow
494 grasses. *Bioresource Technology* 102, 7835–7839.

495 Ranjan, A., Moholkar, V.S., 2013. Comparative study of various pretreatment
496 techniques for rice straw saccharification for the production of alcoholic biofuels.
497 *Fuel* 112, 567–571.

498 Rodriguez, C., Alaswad, A., Benyounis, K.Y., Olabi, A.G., 2016. Pretreatment

499 techniques used in biogas production from grass. *Renewable and Sustainable*
500 *Energy Reviews* 1–12.

501 Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D.,
502 2011. Determination of Structural Carbohydrates and Lignin in Biomass. Golden,
503 CO.

504 Tedesco, S., Mac Lochlainn, D., Olabi, A.G., 2014. Particle size reduction optimization
505 of *Laminaria* spp. biomass for enhanced methane production. *Energy* 76, 857–862.

506 Triolo, J.M., Sommer, S.G., Møller, H.B., Weisbjerg, M.R., Jiang, X.Y., 2011. A new
507 algorithm to characterize biodegradability of biomass during anaerobic digestion:
508 influence of lignin concentration on methane production potential. *Bioresource*
509 *technology* 102, 9395–402.

510 Tsapekos, P., Kougias, P.G., Angelidaki, I., 2015. Biogas production from ensiled
511 meadow grass; effect of mechanical pretreatments and rapid determination of
512 substrate biodegradability via physicochemical methods. *Bioresource Technology*
513 182, 329–335.

514 Tsapekos, P., Kougias, P.G., Egelund, H., Larsen, U., Pedersen, J., Trénel, P.,
515 Angelidaki, I., 2017a. Mechanical pretreatment at harvesting increases the
516 bioenergy output from marginal land grasses. *Renewable Energy* 111, 914–921.

517 Tsapekos, P., Kougias, P.G., Egelund, H., Larsen, U., Pedersen, J., Trénel, P.,
518 Angelidaki, I., 2017b. Improving the energy balance of grass-based anaerobic
519 digestion through harvesting optimisation. *Anaerobe* 46, 131–137.

520 Vervaeren, H., Hostyn, K., Ghekiere, G., Willems, B., 2010. Biological ensilage
521 additives as pretreatment for maize to increase the biogas production. *Renewable*
522 *Energy* 35, 2089–2093.

523 Wang, H., Lehtomäki, A., Tolvanen, K., Puhakka, J., Rintala, J., 2009. Impact of crop
524 species on bacterial community structure during anaerobic co-digestion of crops
525 and cow manure. *Bioresource Technology* 100, 2311–2315.

526

527 **Figure captions**

528 **Fig. 1.** Comminuting machines used for the mechanical pretreatment of grass.

529

530 **Fig. 2.** Accumulated methane yields of untreated and mechanically pretreated grass.

531

532 **Fig. 3.** SEM images of meadow grass particles untreated (a) and pretreated with the
533 initial (b) and modified (c) pretreatment unit

534

535 **Fig. 4.** Correlation loadings plot for the first principal component (PC1) versus the
536 second principal component (PC2) of structural polymers.

537

539 **Table 1.** Chemical composition and methane yield of used lignocellulosic substrates

	Glucan, %VS	Xylan, %VS	Arabinan, %VS	Lignin, %VS	Protein, %VS	C:N ratio	CH₄ yield, mL/gVS
M1	48±2	24±1	6±0	20±1	3±0	24±1	474±50
M2	37±3	24±1	4±0	41±0	2±0	21±1	317±12
M3	36±1	22±1	4±0	37±2	2±0	25±0	307±25
M4	37±0	21±0	4±0	36±1	2±0	23±0	305±15
M5	40±0	24±0	5±0	29±0	2±0	24±1	292±15
M6	49±2	20±1	5±1	25±0	2±0	19±1	292±23
M7	42±1	29±1	5±1	23±1	1±0	25±1	299±35
GC	54±4	21±2	7±1	15±5	3±0	14±0	326±43
WS	40±2	27±1	3±0	30±3	0±0	103±5	255±17
DB	34±0	17±0	1±0	47±0	1±0	45±2	42±8
MIN	33.5	16.4	1.1	9.6	0.2	13.3	33.9
MAX	58.6	29.4	7.2	47.7	3.3	108.5	524.2
MEAN	41.4	22.5	4.3	30.0	1.7	32.2	290.8
SDTEV	6.5	3.5	1.6	9.4	0.8	25.3	104.0
CV	16%	15%	38%	31%	48%	79%	36%

M1-M7: meadow grass; GC: grass clippings; WS: wheat straw; DB: digested biofibers

SDTEV: standard deviation; CV: coefficient of variation

541 **Table 2.** Effects of mechanically pretreatments and summary of kinetic study

<i>1st test</i>	Road sweeping brush against crimper						
	Brush, rpm	Crimper, rpm	CH ₄ yield, mL/gVS	R_{max} , mL/gVS/d	λ , d	R^2	RMSE
T1_0	-	-	305±36	30	3.35	0.999	3.43
T1_a	200	1200	341±21	34	3.17	0.999	4.57
T1_b	600	1200	367±27	31	2.69	0.995	8.90
T1_c	900	1200	344±10	30	2.82	0.997	6.82
T1_d	1200	1200	345±11	31	3.29	0.998	5.97

<i>2nd test</i>	Stable coarse shell against rotated corrugated roller (~400 rpm)						
	Biomass load, kg grass/m ²	CH ₄ yield, mL/gVS	R_{max} , mL/gVS/d	λ , d	R^2	RMSE	
T2_0	-	297±21	31	2.10	0.981	14.67	
T2_a	1.40	364±20	26	1.85	0.988	14.68	
T2_b	1.05	371±20	44	1.83	0.999	4.22	
T2_c	0.80	371±18	39	1.20	0.989	14.01	
T2_d	0.60	361±14	35	1.74	0.996	8.10	
T2_e	0.40	376±28	27	1.53	0.994	10.60	

T1_0 and T2_0: Untreated samples at the first and second test, respectively

T1_a - T1_d and T2_a - T1_e: Mechanically pretreated samples at the first and second test, respectively

R_{max} : maximum CH₄ production rate; λ : lag phase; R^2 : coefficient of determination; RMSE: root mean square error

543 **Table 3.** Pearson's correlation coefficients of chemical compounds and methane yield
 544 of all lignocellulosic samples

Variables	Glucan	Xylan	Arabinan	Lignin	Protein	CH₄ yield
Glucan	1	0.76	0.54	0.04	-0.24	0.04
Xylan		1	0.36	0.32	-0.54	0.01
Arabinan			1	-0.57	0.46	0.56
Lignin				1	-0.87	-0.71
Protein					1	0.62
CH₄ yield						1

545