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Impact of mechanical, chemical and enzymatic pre-treatments on the methane yield from the anaerobic digestion of switchgrass

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ABSTRACT

The conversion of cellulosic crops into biofuels, including methane, is receiving a lot of attention lately. *Panicum vergatum*, or switchgrass, is a warm season perennial grass well adapted to grow in North America. Different pre-treatments were tested in 0.5 l batch reactors, at 35 °C, in order to enhance the methane production from switchgrass, including temperature, sonication, alkalization and autoclaving. The methane production on the basis of volatile solids (VS) added to the fermentation were 112.4 ± 8.4 , 132.5 ± 9.7 and 139.8 ml g^{-1} after 38 days of incubation for winter harvested switchgrass (WHS) after grinding, grinding with alkalization, and grinding with alkalization and autoclaving, respectively. The methane production was higher for fresh summer harvested switchgrass (SHS), with a production of $256.6 \pm 8.2 \text{ ml g}^{-1}$ VS after mulching, alkalization and autoclaving. The methane production from SHS was improved by 29 and 42% when applying lignin (LiP) or manganese peroxidase (MnP), at 202.1 ± 9.8 and $222.9 \pm 22.5 \text{ ml g}^{-1}$ VS, respectively. The combination of an alkali pre-treatment with the MnP increased the methane production furthermore at 297.7 ml g^{-1} VS. The use of pectinases without chemical pre-treatment showed promising yields at 287.4 and 239.5 ml g^{-1} VS for pectate-lyase and poly-galacturonase, respectively. An estimation of the methane yield per hectare of crop harvested resulted in net energy production of 29.8, 49.7 and 78.1 GJ for winter harvested switchgrass, mulched and pretreated summer harvested switchgrass, respectively. Switchgrass represents an interesting candidate as a lignocellulosic crop for methane production.

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1. Introduction

The conversion of cellulosic crops into biofuels is receiving a lot of attention lately, as a second generation energy crops for renewable energy production. Although the concept of biorefinery is mostly applied to the production of ethanol for now, a biorefinery directed toward the production of methane through anaerobic digestion would generate potentially more renewable energy and deserve to be more carefully evaluated [1,2]. In effect, biogas production from energy crops represents a more thermodynamically efficient option than converting

plant matter into liquid fuels [3] and biomethane obtained from anaerobic digestion is the most efficient and clean burning biofuel which is available today [4]. The use of energy crops, mainly corn silage, is also widely spread in the farm-scale anaerobic digesters treating manure, to increase the methane yield and the resulting net renewable energy production [5,6].

Panicum vergatum, or switchgrass, is a high yielding (13–18 tonnes(t) ha⁻¹ in Southeastern United States), warm season, perennial grass that can grow more than 2.75 m in height [7]. It was chosen as the model lignocellulosic crop by the US Department of Energy in the 90s and is believed to

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return 540% more renewable energy than fossil fuel consumption [8], compared to 25% for maize [9]. Switchgrass is also well adapted to grow in Canada, with low fertilizer applications and high resistance to naturally occurring pest and diseases [10]. Switchgrass is highly adaptable to poor soil which makes it an excellent choice since dedicated bioenergy crops would preferably be cultivated on lands not used for primary food [11]. It is mostly used for bedding and combustion as of now in Canada [3]. Switchgrass is composed of around 12–19% lignin, 31–37% hemicellulose and 29–45% cellulose [12,13], hence suggesting a high potential conversion of the plant into biofuel, despite the fact that lignin is poorly degraded under anaerobic conditions [14].

The evaluation of methane production as renewable energy from energy crops or agricultural wastes is not new [15–17] and is being prompted these recent years for the same reasons as 30 years ago: an expected energy shortage, or at least significant price increase, which leads deciders to lean toward other form of energy than fossil fuel. The methane potential of a variety of cellulosic crops have been recently evaluated [14,18–20], but switchgrass has not yet been subjected to such assays to our knowledge, besides a co-digestion study in batch mode with different animal manures [21].

The main challenge of using cellulosic crops for biofuel production is their structure and composition, with layers of lignin linked to cellulose and hemicellulose, preventing enzymatic hydrolysis [18,22]. Therefore, a pre-treatment is needed to disrupt the structure of the crop and allow for a more complete methanisation. A wide spectrum of methods, such as acid hydrolysis, ammonia fiber explosion (AFEX) and steam explosion, are typically employed for the pre-treatment of lignocellulosic crops as reviewed by Hendriks and Zeeman [23] and Sun and Cheng [13]. More recent methodologies include hydrothermolysis at 200 °C [24] or microwave-assisted alkali pre-treatment [25]. Although these methods are efficient at solubilizing the crop, they are energy intensive, and one should be careful as not to use a pre-treatment that costs more energy than it can provide. Furthermore, pre-treatments aimed at the ethanol production route discard the liquid fraction that contains xylose-rich hemicellulose which would be preferable to keep for increased methane production during anaerobic digestion. Biological treatments, either with microorganisms, or with enzymes, are simple and do not require major capital investments, although the increase in biogas yield have been low so far [14]. Thermochemical pre-treatments (alkalis, autoclaving) are promising [18,26] but did not result in significant increase in methane production for the energy crops that were tested. Our objectives were thus to assess the impact of mild pre-treatments on the solubilization of switchgrass, and then evaluate the increase in methane production following anaerobic digestion.

2. Materials and methods

2.1. Switchgrass

The switchgrass was obtained from Ferme Norac (45°16′34.77″ N, 74°0′54.29″ W), and was a Kanlow variety, from an 11 year

old field yielding 10–12 t ha⁻¹. The winter harvested switchgrass (WHS) was cut during Fall 2006, left on the field, and harvested in March 2007. The harvesting of 50 kg of switchgrass was performed manually with garden scissors by cutting the plants at their base. The harvested switchgrass was transported to the laboratory within 4 h of harvesting in large plastic bags and reduced in size with scissors. At this stage, it had a humidity level of 4%. The pieces of WHS were stored in 20 l plastic containers that were flushed with nitrogen gas and kept at 4 °C until use. The summer harvested switchgrass (SHS) was harvested at the end of August 2007 while it was mature and still fully green, and around 2.5–3 m high. At this stage, it had a humidity level of 57%. It was transported to the laboratory within 4 h of harvesting, reduced in pieces of 5–10 cm with scissors and stored in sealed transparent plastic bags that were flushed with nitrogen and then tightly packed to minimize the amount of void in the bags. The bags were kept a 4 °C until use.

The Table 1 is showing a characterization of both switchgrass. It has to be noted that the carbon to nitrogen and carbon to phosphorous ratio were more favorable for the SHS than for the WHS, when compared with suggested C:N ratio of 25:1 to 40:1, and C:P ratio of 200:1–600:1 [27–29]. The carbohydrates were determined for the SHS and expressed in dry kg of substrate. The resulting concentrations were 2.4, 18.5, 0.5, 1.3 and 39.4 g kg⁻¹ for the arabinan, xylan, mannan, galactan and glucan, respectively.

2.2. Description of the pre-treatments applied to switchgrass

A mechanical pre-treatment was applied to both harvest of switchgrass as a first step prior to the other pre-treatments. The WHS was ground to a powder with a sample mill Foss tecator model Cyclotec 1093 (Hoganas, Sweden). Specifically, WHS was further cut in pieces of around one to three cm long and a sample of 10 g was inserted in the mill. Grinding was performed until the sample was completely under a powder form. The SHS was cut in small pieces (e.g. mulched) varying

Table 1 – Characterization of the switchgrass used in the assays.

Parameters	WHS	SHS
Total solids (TS)	961 ± 2	427 ± 67
Volatile solids (VS)	934 ± 2	396 ± 64
Nitrogen	1.830	6.110
Phosphorous	0.382	0.901
Potassium	ND	9.460
Ratio C:N	491:1	92:1
Ratio C:P	2344:1	624:1
Lignin total	22.3% TS	28.1% TS
acid-insoluble	20.7	24.4
acid-soluble	1.6	3.7
Hemicellulose	ND	37.4% TS
Cellulose	ND	24.6% TS

TS, VS, N, P and K in g kg⁻¹.

WHS: winter harvested switchgrass; SHS: fresh summer harvested switchgrass ND: not determined.

between 0.2 and 1.0 cm with a knife mill Grindomix model GM200 (Newton, PA), with the blades spinning at 83 Hz for 20 s.

Different pre-treatments were then tested on both harvest of switchgrass: temperature, sonication, alkalization, combined temperature and alkalization, autoclaving (temperature and pressure), combined alkalization and autoclaving. The temperature pre-treatment consisted in incubating an aliquot of switchgrass suspension in a water bath maintained at 90 °C for 3 h. The sonication was performed with a Vibra Cell VC130 sonicator from Sonics (Newton, CT) working at a frequency of 20 kHz, using an amplitude of 40% with a 2 s pulse. Alkalization was performed with a solution of NaOH. A concentration of 7 g l⁻¹ of NaOH was added to the switchgrass, as used by Kim et al. [30] for municipal sludge pre-treatment. This high concentration was preferred over lower concentration with higher incubation time [31,32]. The switchgrass was then incubated for 3 h, at 35 or 55 °C. Autoclaving was done in a Steris SV-120 autoclave, at 121 °C and 142 kPa for 15 min, as performed elsewhere [30,33]. The combination of autoclaving and alkalization was performed as mentioned in Kim et al. [30] and Valo et al. [34]. The pre-treatments were tested on a suspension of the switchgrass, made from adding 90 ml of distilled water to 10 g (wet) of either WHS or SHS for resulting moisture level of 90 and 96%, respectively. The solubilization of the volatile suspended solids (VSS), and the increase in soluble chemical oxygen demand (COD) and acetate were chosen as indicators for the hydrolysis of the samples and to compare the relative efficiencies of the pre-treatments.

2.3. Preparation of the methane potential assays

The methane potential assays with the WHS were prepared based on the Biochemical Methane Potential (BMP) assay for wastewater from Cornacchio et al. [35]. A few modifications were done to adapt the test to solid samples. A total of 10 g of WHS was suspended in 80 ml of water and 5 ml each of an urea solution (8 g l⁻¹) and KH₂PO₄ solution (6.5 g l⁻¹) in order to compensate for the low C:N and C:P ratio. The pre-treatments were performed on the suspension prior to the addition in the bottle. The inoculum consisted in 20 g of granular biomass collected from a full scale upflow anaerobic sludge blanket (UASB) digester treating apple processing wastewater (Lassonde Inc., Rougemont, QC, Canada; 45°25'52.71" N, 73°03'12.15" W), for a resulting moisture level of 90%. The inoculum was starved for 48 h prior to the start-up of the assays, by being incubated at 35 °C and an agitation at 2 Hz with no substrate.

The defined media was concentrated 5 times compared to the original solution and 2 ml were added to each bottle. It contained 50 ml l⁻¹ of mineral solution I (g l⁻¹) (50 NaCl; 10 CaCl₂·H₂O; 189.4 NH₄Cl; 10 MgCl₂·6H₂O), 5 ml l⁻¹ of mineral solution II (g l⁻¹) (10 (NH₄)₆Mo₇O₂₄·4H₂O; 0.1 ZnSO₄·7H₂O; 0.3 H₃BO₃; 1.5 FeCl₂·4H₂O; 10 CoCl₂·6H₂O; 0.03 MnCl₂·4H₂O; 0.03 NiCl₂·6H₂O; 0.1 AlK (SO₄)₂·12H₂O), 5 ml l⁻¹ of vitamin B solution (g l⁻¹) (0.1 nicotinic acid; 0.1 cyanocobalamin; 0.05 thiamin; 0.05 p-aminobenzoic acid; 0.25 pyridoxine; 0.025 pantothenic acid), 50 ml l⁻¹ of a phosphate solution (50 g l⁻¹ KH₂PO₄), 1.5 ml of a resazurin solution (0.1 g l⁻¹), 5 ml of a 2-methyl-butyric acid solution (102 g l⁻¹). 2 ml of bicarbonate buffer, made from

42 g l⁻¹ NaHCO₃ and 100 g l⁻¹ KHCO₃, along with 0.5 ml of 1.25% Na₂S-cysteine solution, completed the preparation for each bottle. The final liquid volume was around 125 ml.

The bottles were prepared anaerobically in triplicates for each experiment, in 500 ml serum bottles and filled under a constant nitrogen flow. The pH was adjusted if necessary when the bottles were completed, before sealing. During the filling of the bottles, aliquots of the added inoculum were sampled and analyzed for VSS concentration, to insure that a constant amount of inoculum was added through the experiment. A fourth bottle was prepared as well, and was used to obtain initial characterization of the assay, namely pH, VSS, soluble chemical oxygen demand (sCOD) and volatile fatty acids (VFA). The bottles were incubated at 35 °C with an agitation of 2.5 Hz. Control bottles were prepared to allow the removal of endogenous methane production from the assays. The control bottles were identical to the test bottles, excepted that the WHS or SHS suspension were replaced with the same volume of deoxygenated water. The assays were conducted until the methane production became negligible (<5 ml d⁻¹) as performed elsewhere [36].

The methane potential assays with the SHS were prepared as for the WHS assays, with these modifications. Five grams of SHS was suspended in 80 ml of water and added in the bottles for a resulting moisture level of 96%. The pre-treatments were performed on the suspension prior to the addition in the bottle. As for the inocula, it consisted in a mix from two different sources. Granular biomass was collected from a full scale anaerobic digester treating fruit processing wastewaters. Rumen was collected in a slaughterhouse from a recently killed cow. The inoculum was prepared with a ratio of 80% granular biomass and 20% rumen on a solid basis by adding 5 g of each in the bottles. The ratio of inoculum to sample was also modified compared to the original method, at 1:2 for the winter harvested switchgrass assays, and 1:1 for the assays on the summer harvested switchgrass, as reported by Lehtomäki et al. [36] for grass silage methane potential assays.

2.4. Enzymatic pre-treatments for the methane potential assays

Four enzymes were tested as pre-treatments prior to the methane potential assays on summer harvested switchgrass: two peroxidases, lignin peroxidase (LiP) and manganese peroxidase (MnP); and two pectinases, pectate-lyase (PL) and poly-galacturonase (PG). The enzymatic methane potential assays were prepared differently from the other assays, due to the particular preparation protocol for the application of the enzymes. The summer harvested switchgrass was mulched as described in Section 2.2 prior to the enzymatic pre-treatment.

The lignin peroxidase (LiP) was purchased from Sigma–Aldrich and required cofactors for its activation. The pre-treatment was performed in small batches containing 2 wet grams of mulched switchgrass in a total volume of 40 ml, agitated at 2.5 Hz and incubated at 22 °C, in a sealed vial for 8 h. Forty enzyme units (U, 1 μmol min⁻¹ or 16.67 nkat) were added (1 U ml⁻¹), along with the following cofactors: sodium tartrate (pH 4.5, 15 mmol l⁻¹), veratryl alcohol (2.5 mmol l⁻¹), H₂O₂ (0.33 mmol l⁻¹) and oxalate (0.2 mmol l⁻¹) as suggested in Stahl et al. [37] and Fournier et al. [38]. The manganese

peroxidase (MnP) was purchased from Jena Bioscience GmbH (Germany) and also required cofactors for its activation. The pre-treatment was performed in small batches containing 2 wet grams of mulched switchgrass in a total volume of 40 ml, agitated at 2.5 Hz and incubated at 37 °C, in a sealed vial for 8 h. A loading of 80 U (2 U ml⁻¹) of the enzyme was added, along with the following cofactors: sodium malonate (pH 4.5, 50 mmol l⁻¹), MnCl₂ (2 mmol l⁻¹), H₂O₂ (0.33 mmol l⁻¹) and 0.5% Tween 80, as suggested in Horfrichter et al. [39] and Fournier et al. [40]. The LiP and MnP pre-treatments were also verified coupled to an alkaline pre-treatment. For these assays, the enzymatic pre-treatment was applied first, then alkalization was performed as described previously. A total of 10 g instead of 20 g of inoculum was added to the methane potential assays, since only 2 g of SHS were used instead of 5 g as for the previous assays, for a resulting moisture level of 97%. Endogenous control bottles were prepared as in Section 2.3. Additionally, control bottles were prepared with inoculum and the cofactors that were required for the activation of the enzymes. These controls were prepared in order to quantify a potential methane production from the degradation of the cofactors.

The poly-galacturonase (PG) was obtained from Dr Lau's laboratory (NRC, BRI) as described in Xiao et al. [41] and displayed an activity of 8 U ml⁻¹. The application on the SHS was performed at pH 5, adjusted with glacial acetic acid, in 20 mmol l⁻¹ of sodium acetate. The SHS preparation was then incubated at room temperature overnight, prior to the methane potential assay. Two different loading of the enzyme were tested, by mixing 1 or 5 ml of the PG with the SHS, for loadings of 10 and 50 U g⁻¹ VSS added. The pectate-lyase (PL) was also obtained from Dr Lau's laboratory as described in Xiao et al. [41] and showed an activity of 1000 U ml⁻¹. The application on the SHS was performed at pH 8.5, in 20 mmol l⁻¹ Tris–HCl buffer. As for the PG assay, the SHS preparation was incubated at room temperature overnight, prior to the methane potential assay. Three different loading of the enzyme were tested, by mixing 1, 2 or 5 ml of the PG with the SHS, for loadings of 1263, 2525 and 6313 U g⁻¹ VSS added. Finally, a sequential pre-treatment was tested by applying PG, then PL on the same sample of SHS prior the methane potential assay, at loadings of 1 and 5 ml. All assays were prepared in single bottles instead of triplicates, since the amount of available enzyme was limited. Different control bottles were prepared: an endogenous control containing only the inoculum, a control containing the inoculum with the buffer used for the enzymatic pre-treatment and a control with the SHS without enzymatic pre-treatment. The incubation and monitoring of these enzymatic assays were done under the same conditions as for the other pre-treatment assays.

2.5. Analytical methods

The biogas production was measured in the batch reactors with a water-displacement system build from a volumetric glass burette, with graduation every 0.2 ml. All gas or methane volumes presented in this study are described at standard temperature and pressure, e.g. 273.15 K and 100 kPa pressure. After equilibrium of the bottle headspace to ambient pressure, a gas sample (0.3 ml) was taken with a model 1750 gas-tight

syringe (Hamilton, Reno, USA) and analyzed for H₂, N₂, CH₄ and CO₂ by gas chromatography (GC). The GC was a Agilent 6890 (Agilent Technologies, Wilmington, USA) coupled to a thermal conductivity detector (TCD). The gas sample was injected on a 11 m × 2 mm I.D. Chromosorb 102 packed column (Supelco, Bellefonte, USA). The column was heated at 50 °C and maintained for 3.9 min. Argon was used as the carrier gas. The injector and detector were maintained at 125 °C and 150 °C, respectively.

Liquid samples (1.5 ml) were taken from the serum bottles with 3 ml syringes equipped with a number 26 gauge needle and analyzed for pH, soluble chemical oxygen demand (COD) and volatile fatty acids (VFA), namely acetate, propionate and butyrate. Total volatile solids (TVS) and VSS were also determined at the beginning of the assays and at the end for each bottle. The pH was measured on an Accumet AP61 portable pH meter equipped with a micro probe (Fisher, Fairlawn, USA) directly on the recuperated sample, within 1 min of sampling. The COD and the VSS were determined according to Standard Methods [42].

The samples for the volatile fatty acids were centrifuged at 12 100g in a J2-21M centrifuge (Beckmann, Canada) equipped with a JA-20 rotor for 10 min. A volume of 350 µl of the supernatant was then mixed with an internal standard containing 6% formic acid and injected in the GC. The analysis were performed on a Perkin–Elmer Sigma 200GC (Norwalk, USA) equipped with a flame-ionization detector (FID). A sample volume of 0.5 µl was injected on a 3 ft × 2 mm 60/80 Carbowax C column with 0.3% Carbowax 20 M and 0.1% H₃PO₄ (Supelco, Bellefonte, USA). The oven, injector and detector temperatures were 120 °C, 200 °C and 200 °C, respectively, and the method lasted 4 min. Nitrogen was used as the carrier gas at a flowrate of 50 ml min⁻¹.

The lignin content was determined according to PAPTAC testing methods G8 and G9 (Paprican, Pointe – Claire, Canada). The carbohydrate composition was determined according to TAPPI test method T249- cm-85 (Paprican, Pointeclaire, Canada). The hemicellulose and cellulose percentage were estimated from the sugars concentration. Namely, the glucose contribution to the hemicelluloses was estimated from a ratio of 2:1 for mannose: glucose and 10:1 for xylose: glucose. The cellulose fraction was then calculated by difference between total glucose and glucose associated with hemicelluloses.

3. Results and discussion

3.1. Pre-treatments impact on the solubilization of winter and summer harvested switchgrass

The Table 2 presents the results obtained for the different pre-treatments that were tested on both harvest of switchgrass. The pre-treatments had a greater impact in general on the summer harvest switchgrass compared with the winter harvest. Temperature, sonication and autoclaving had no significant impact on the WHS, as shown by the similar VSS and sCOD concentration before and after pre-treatment, and only a slight impact on the SHS. The alkalization at 35 °C resulted in the solubilization of 8 and 22% of the VSS for the WHS and SHS, respectively, achieving a 3-fold and 5-fold

Table 2 – Impact of the pre-treatments on the solubilization of the switchgrass.

Pre-treatment	Parameter	Winter harvest		Summer harvest	
		Initial	Final	Initial	Final
Temperature	VSS	97.0	93.0	43.0	34.4
	sCOD	8.0	6.0	0.7	7.8
	Acetate	65	120	0	54
Sonication	VSS	97.0	98.0	43.0	41.9
	sCOD	8.0	5.0	0.7	4.8
	Acetate	65	68	0	59
NaOH, 35 °C	VSS	97.0	89.0	38.1	29.1
	sCOD	8.0	22.0	1.9	10.1
	Acetate	65	3435	214	1204
NaOH, 55 °C	VSS	97.0	88.0	38.1	29.2
	sCOD	8.0	28.0	1.9	14.1
	Acetate	65	3410	214	1123
Autoclave	VSS	97.0	103.0	43.0	35.7
	sCOD	8.0	7.0	0.7	7.0
	Acetate	65	40	0	51
NaOH–Autoclave	VSS	97.0	80.0	38.0	21.8
	sCOD	8.0	41.0	2.0	24.0
	Acetate	65	3090	214	1095

VSS, sCOD in g l⁻¹ of suspension. VFA in mg l⁻¹ of suspension. The suspension was prepared from 10 g of wet WHS or SHS and 90 ml of water.

increase of the sCOD (Table 2). The alkalization at a higher temperature (55 °C), resulted in 27 and 40% higher sCOD than for the pre-treatment at 35 °C. Finally, the best pre-treatment applied to both harvest was the combination of alkalization and autoclaving. The VSS solubilization reached 18 and 43% with 5 and 20 times more sCOD after the pre-treatment for the WHS and the SHS, respectively. In regard of those results, the alkalization at 35 °C and the combination of alkalization and autoclaving were chosen as chemical pre-treatment for the evaluation of the methane potential from WHS and SHS.

3.2. Methane potential assays for the WHS

The Table 3 is showing the initial and final values for the three pre-treatment assays. The grinding had little impact on the solubilization of organic material from the WHS as shown by the low initial sCOD and VFA concentration. The sCOD concentration was higher at the end of the incubation time but it can be presumed that this was recalcitrant or non biodegradable material given the low VFA concentration. The VSS concentration was 32, 40 and 48% lower at the end of the assay, although this cannot be directly used to calculate the degradation percentage, since a fraction of the VSS consisted in the inoculum.

The assay with NaOH pre-treatment displayed a partial solubilization of the WHS with 18.2 g l⁻¹ and around 3 g l⁻¹ of VFA after pre-treatment. The concentration of soluble COD remained high at the end of the assays, with low VFA concentration. This would suggest that the NaOH pre-treatment solubilized an important fraction of non biodegradable organic matter. This hard sCOD is presumed to be lignin, as it was shown that alkalization pre-treatment attacks crops by solubilizing the lignin [43,44]. The grinding, NaOH and

Table 3 – Operational parameters for the methane potential assay with WHS and SHS.

Assays	VS _i	VS _f	sCOD _i	sCOD _f	VFA _f
WHS grinded	83.6	56.9	2.9	5.9	70
WHS grinded-NaOH	86.3	51.6	18.2	13.4	223
WHS grinded-NaOH-alk	86.5	44.9	27.4	29.2	3684
SHS chopped	32.2	26.6	0.4	0.8	0
SHS mulched	31.7	26.5	0.8	1.4	0
SHS NaOH-alk	26.4	18.5	4.8	3.7	16

i: initial; f: final.
VS in g kg⁻¹ and sCOD in g l⁻¹; VFA in mg l⁻¹.
Values were obtained from pooled aliquots from the triplicate of bottles.

autoclaving pre-treatment yielded the higher amount of soluble material with 27.4 and over 3 g l⁻¹ for the soluble COD and VFA initial concentration, respectively. There was still a significant concentration of acetate and propionate at the end of the assay, indicating the inhibition of the anaerobic consortia during the course of the incubation time. The sCOD concentration was also as high at the end of the assay, compared to the initial time (Table 3). A possible explanation for this inhibition could be the presence of furfurals and phenols in the bulk liquid, deriving from the hydrolysis of the switchgrass during pre-treatment [23,45].

The resulting methane production for the three different experiments with the WHS were 112.4 ± 8.4 ml g⁻¹ VS (ground WHS), 132.5 ± 9.7 ml g⁻¹ VS (ground with alkalization), and 139.8 ml g⁻¹ VS (ground, alkalization and autoclaving), as shown in Fig. 1. The assays were incubated during 38 days. The alkalization and combined alkalization and autoclaving of the ground WHS thus increased the methane production by 18% and 24% compared to the mechanical pre-treatment alone. These results are similar to those obtained after an alkali pre-treatment (+17%) for grass hay [18]. The

Fig. 1 – Methane production from the pretreated WHS and SHS. Methane from the endogenous controls was subtracted. WHS: winter harvested switchgrass. SHS: summer harvested switchgrass. Alk: alkalization pre-treatment. Auto: autoclaving pre-treatment.

variances of the average methane production from these three assays were compared to each other, followed by a t-test analysis for two samples, in order to determine if the methane production from the pretreated WHS was significantly different from the untreated WHS assay. The t-test was performed with an alpha of 0.1 and the results for P were 0.049, 0.068 and 0.160 for the following pairs: 1) ground WHS and ground WHS with alkalization; 2) ground WHS and ground WHS with alkalization and autoclaving; 3) ground WHS with alkalization and ground WHS with alkalization and autoclaving. Therefore, there was a significant difference in the methane production between the untreated and pretreated WHS, but not between the two pre-treatments. Although the methane production from WHS remained low even after a combined mechanical and chemical pre-treatment, most of the methane was expressed in the first three weeks of incubation with 93–95% of the total methane production. Still, the total methane production remained low compared with similar feedstock, such as straw of oats and straw of rapeseed, which yielded 0.17–0.24 l g⁻¹ VSS after 50 days of incubation [36].

A balance can be performed in order to estimate the WHS mineralization from the methane produced compared with the solids degradation. The COD concentration of the WHS was measured at 1.262 g g⁻¹ VS and 350 ml of methane at standard temperature and pressure is stoichiometrically generated for each gram of COD degraded. The mineralization of the WHS reached 25, 30 and 32% for the grinded WHS, grinded with alkalization, and grinded, alkalization and autoclaving, when calculated from the methane produced in the assays. The 10 g of WHS added represented 9.33 g VS added, from which 23% is lignin, known to be refractory and poorly degraded under anaerobic conditions [46]. In effect, one has to be cautious with the VS concentration since it is not the best measure of digestible fraction of crops as it includes lignin [47]. Thus, 33, 39 and 41% of the biodegradable VS (without lignin) of the WHS was mineralized for the grinded, alkalization, alkalization and autoclaving assays, respectively. This is less than the 59% VS reduction obtained from Lehtomäki and Björnsson [48] with grass silage, although the lignin content was much lower for their substrate at 5.4%.

The rather low conversion of WHS into methane could be explained by its dryness. In effect, it was showed that drying of a substrate severely reduces the digestibility of the cellulose, even after rehydration [49]. Also, drying of the switchgrass could have affected the structure and the crystallinity index of the cellulose, in a way that it would prevent enzymatic degradation by anaerobic consortia [46]. It was also demonstrated that this reaction was irreversible.

3.3. Methane potential assays for the mechanically and chemically pretreated SHS

The methane potential assay was performed on summer harvested switchgrass under three different pre-treatments. In order to really assess the benefit of a thorough mechanical pre-treatment, such as mulching, a second set of assay was prepared with simply chopped SHS, in pieces of roughly one to three cm. The SHS was also tested after alkalisation combined with autoclaving, as for the WHS.

The Table 3 is showing the initial and final values for the SHS assays. The mechanical pre-treatments, chopping or mulching, had little impact on the solubilization of organic material from the SHS as shown by the low initial sCOD and VFA concentration (<30 mg l⁻¹), while the alkalization and autoclaving pre-treatment achieved a partial solubilization, around 20%, along with the generation of 416 mg l⁻¹ acetate and 169 mg l⁻¹ propionate. The sCOD concentration remained at similar concentration at the end of the assays, compared to the initial time, but no VFA were measured. This would suggest the presence of non biodegradable organic material, as discussed in the WHS section, but to a much lesser extent. The VSS concentration at the end of the assays were similar for the chopped and mulched assays which is in discordance with the amount of methane produced. However, an intermediate VSS sampling at day 18 showed a concentration of only 23.6 g l⁻¹ for the mulched and alkalized SHS, compared with 26.5 g l⁻¹ for day 36. Therefore, the final VSS concentration for this assay should be disregarded.

The 36 days of incubation resulted in a final net methane production of 94.7 ± 4.4, 152.3 ± 1.2 and 256.6 ± 8.2 ml g⁻¹ VS, for the chopped, mulched and alkalized and autoclaved SHS, respectively (Fig. 1). Thus, a thorough mechanical pre-treatment was efficient at increasing the methane produced from the SHS, with 61% more methane generated from the mulched SHS compared with the chopped SHS. Mulching of the SHS was much more beneficial for the methane production than what was reviewed for mechanical pre-treatment (milling) [23], with reported increase in methane from 5 to 25% coupled to reduction in the time duration of the incubation by 23–59%. Mechanical pre-treatments could thus be more critical for fresh feedstock digestion, and possibly ensiled material as well, compared to dried feedstock. A chemical pre-treatment could further enhance the methane production by 68%, e.g. close to three times more methane than for the chopped switchgrass, as shown with the alkalization and autoclave pre-treatment.

3.4. Methane potential assays for the enzymatically pretreated SHS

3.4.1. Lignin and manganese peroxidase pre-treatment

The LiP and MnP are enzymes that degrade lignin. Their potential positive action on the anaerobic digestion of pretreated SHS could be by physically removing the lignin and allowing enzymatic hydrolysis of the hemicellulose and cellulose, or by degrading and solubilizing lignin to a point where it could be degraded.

The Table 4 is showing the initial and final values for the LiP and MnP assays. Endogenous control bottles were prepared for each set of assays. The pH remained at a neutral value for all assays (7.08–7.42). The VSS concentration was reduced by more than half in all controls, from 11.8 g l⁻¹ to 5.1 ± 0.5, 4.5 ± 1.0 and 4.9 ± 0.1 g l⁻¹ for the control, control LiP and control MnP, respectively (Table 4). The VSS was reduced by 54–55% for the SHS, SHS–LiP and SHS–MnP assays compared to 59–71% for the SHS–Alk, LiP–Alk and MnP–Alk assays. The difference could be attributed to the higher conversion in methane for the Alk assays, but also by the initial solubilization of the switchgrass during alkalization.

Table 4 – Operational parameters for the methane potential assay with enzymatic pre-treatments.

Assays	VS _i	VS _f	sCOD _i	sCOD _f	VFA _f
Control	11.8	5.1 (0.5)	ND	0.7 (0.1)	10
SHS	18.6 (3.9)	8.6 (1.4)	2.4	1.3 (0.1)	10
SHS–ALK	18.8 (3.0)	6.7 (0.6)	3.3	3.0 (0.2)	160
Control LiP	11.8	4.5 (1.0)	ND	0.9 (0.1)	120
LiP	18.6 (3.5)	8.9 (1.0)	2.6	1.2 (0.0)	10
LiP–ALK	17.8 (1.0)	5.6 (0.7)	4.9	2.4 (0.2)	40
Control MnP	11.8	4.9 (0.1)	ND	2.6 (0.2)	10
MnP	17.5 (2.7)	8.9 (0.7)	3.3	2.6 (0.1)	0
MnP–ALK	18.7 (1.0)	7.9 (1.0)	4.2	3.9 (0.4)	20
SHS + tris–HCl	34.4	18.8	6.8	4.4	50
SHS–PL 1263 U	33.7	15.4	6.9	4.4	10
SHS–PL 2525 U	32.5	14.7	6.6	5.1	10
SHS–PL 6313 U	34.8	14.8	7.2	5.0	0
SHS + Na–acetate	33	12.4	5.5	3.2	0
SHS–PG 10 U	33.2	16.2	5.6	2.9	0
SHS–PG 50 U	38.7	13.0	5.4	2.8	0

SHS: summer harvested switchgrass.

LiP: lignin peroxidase. MnP: manganese peroxidase. PL: pectate-lyase. PG: poly-galacturonase.

U: enzyme units, 1 $\mu\text{mol substrate min}^{-1}$ or 16.67 nkat
i: initial; f: final.

ND: not determined. VS and sCOD in g l^{-1} ; VFA in mg l^{-1} .

Values in parenthesis represent the standard deviation. Otherwise, values were obtained from pooled aliquots from the triplicate of bottles.

Small concentrations of VFA, mostly acetate, were found at the end of the assays with an alkaline pre-treatment. Propionate was not found in the controls and the assays with enzymes only, but 6 ± 5 , 27 ± 17 and $6 \pm 10 \text{ mg l}^{-1}$ were measured at the end of the assays SHS–Alk, SHS–LiP–Alk and SHS–MnP–Alk, respectively. No butyrate was measured in the final characterization of all assays.

The methane potential assays were monitored for 37 days as for the WHS and SHS assays. At that time, most of the methane production was obtained (80–88% of the final values) excepted for MnP–Alk that still showed significant methane production. The Fig. 2 is presenting the methane potential results with mulched SHS, untreated or enzymatically pre-treated with the LiP or MnP enzymes at the end of the incubation period, which reached 134 days. The final methane production were 157.0 ± 18.3 , 202.1 ± 9.8 and $222.9 \pm 22.5 \text{ ml g}^{-1} \text{ VS}$ for the SHS, SHS with LiP and SHS with MnP assays, respectively.

The Fig. 2 is also presenting the methane potential assays performed with mulched SHS, untreated or enzymatically pretreated with the LiP or MnP enzymes followed with an alkaline pre-treatment. The final methane production were 243.6 ± 0.7 , 254.9 ± 14.0 and $297.7 \text{ ml g}^{-1} \text{ VS}$ after 134 days of incubation, for the SHS–Alk, SHS with LiP–Alk and SHS with MnP–Alk assays, respectively. Again, between 81 and 87% of the methane was produced within the first 37 days of incubation.

The increase in methane production attributed to the combined enzymes and alkaline pre-treatments was only 5 and 22% for the LiP–Alk and MnP–Alk enzymes, respectively. In effect, the combined enzymes and alkalization had a clear positive effect on the methane potential from the SHS

Fig. 2 – Methane production from the SHS with peroxidase pre-treatment. Methane from the endogenous controls (including enzyme and cofactors) was subtracted. SHS: summer harvested switchgrass. Alk: alkalization pre-treatment.

compared with the SHS alone (55% increase) while the difference between the enzymes assays and combined assays was more modest at 26 and 34% for the LiP and MnP, respectively. This would suggest that the positive impact on methane production from the application of enzymes on the SHS was similar to the impact of an alkaline pre-treatment. This is possible considering that the enzymes are degrading lignin, and that the NaOH impact is to dissolve the lignin. Still, this would mean that a biological pre-treatment (enzymes) could provide a similar impact on the methane production of switchgrass and become an alternative to the more conventional chemical pre-treatment. The use of enzymatic breakdown of the lignin could also reduce the risk of liberating inhibitors such as phenolic compounds and furfurals, as observed with alkalis pre-treatments.

3.4.2. Pectinases enzymatic pre-treatment

The pectinases are enzymes that are promising for the retting of bast fibers [41]. Pectate-lyase was efficiently used for bio-scouring as a replacement for sodium hydroxide [50,51]. The pectate-lyase (PL) enzyme required cofactors, including tris–HCl buffer, to react with the substrate. Endogenous control bottles were prepared with the tris–HCl buffer (soluble COD 2.6 g l^{-1}) to account for possible methane production resulting from its degradation.

The Fig. 3 is showing the methane potential assays performed with mulched SHS, untreated or enzymatically pre-treated with the PL enzyme at different loading. The methane production were 205.1, 190.4, 279.2 and $287.4 \text{ ml g}^{-1} \text{ VS}$ after 78 days of incubation, for the SHS with tris–HCl, SHS with 1263, 2525 and 6313 U PL $\text{g}^{-1} \text{ VS}$ added, respectively. The higher loadings of PL (2525 and 6313 U) increased the methane production by 36 and 40%, while the lower loading of PL (1263 U) displayed 7% less methane production in comparison to the endogenous control with SHS and tris–HCl buffer. These positive results are in agreement with Berlin et al. [52]

Fig. 3 – Methane production from the SHS with pectinase pre-treatment. Methane from the endogenous controls (including tris–HCl or sodium acetate buffer) was subtracted. SHS: summer harvested switchgrass. PL: pectate-lyase. PG: poly-galacturonase. U: enzyme unit, 1 $\mu\text{mol substrate min}^{-1}$ or 16.67 nkat.

who suspected that the pectin was limiting the enzyme access to the cellulose and that the use of pectinases could free the cellulose. The current assay would be an indirect confirmation that pectinases helped accessing the cellulose.

The poly-galacturonase (PG) enzyme required cofactors, including sodium acetate. Endogenous control bottles were prepared with the sodium acetate buffer (soluble COD 1.7 g l^{-1}) to account for possible methane production resulting from its degradation.

The Fig. 3 is presenting the methane potential assays performed with mulched SHS, untreated or enzymatically pre-treated with the PG enzyme at two different loading. The methane production were 139.1, 64.9 and 239.5 $\text{ml g}^{-1} \text{ VS}$ after 78 days of incubation, for the SHS with sodium acetate, SHS with 10 and 50 U PL $\text{g}^{-1} \text{ VS}$ added, respectively. The higher loadings of PL increased the methane production by 72%, while the lower loading of PL displayed 53% less methane production in comparison with the control SHS with sodium acetate buffer. It is unlikely that this would be the result of an inhibition from the enzyme since the high enzyme loading has a strong positive impact.

The Table 4 is presenting the initial and final values for the PL and PG assays. Each set of assay had its endogenous control bottles. The pH remained at a neutral value for all assays (7.0–7.3). The initial VSS concentration was $34.7 \pm 2.4 \text{ g l}^{-1}$ which confirmed that the mulched switchgrass was homogenous. The VSS reduction could not always be linked to the methane production for the assays. This could be due partly because part of the remaining VSS in the bottles consisted in the inoculum, and its concentration could evolve differently in the bottles. The VSS was reduced by 55–57% and 51–66% in the assays with the PL and PG enzyme, respectively, compared to 45% and 62% for their respective control assay. The sCOD concentrations were consistently lower at the end of the assays with $39 \pm 8\%$ removal on average (Table 4). Although the initial VFA concentration in COD equivalent were high for all assays with an average of 3031 mg l^{-1} , presumably because of the presence of the organic buffer, it was all degraded during the incubation of the assays, which suggests that there was no inhibition of the degradation of the SHS caused by the presence of the enzymes and cofactors.

The impact of peroxidases or pectinases on the increase in methane production is thus greater than the use of cellulases and hemicellulases, as reported by Lehtomäki et al. [18] who showed an increase of only 17% in methane production from a combined alkalis and enzymatic pre-treatment.

3.4.3. Field yield estimation

The methane production per mass of volatile solids of switchgrass gives an important information, although the specific yield per cultivated hectare can give a more precise indication of the crop potential for biofuel production. A production of $287\text{--}298 \text{ ml g}^{-1} \text{ VS}$ could be obtained from the anaerobic digestion of summer harvested switchgrass, using a preliminary incubation with a combination of pectinases, or a combination of peroxidase and alkalization as pre-treatments. Switchgrass fields in northern climate can yield $10 \text{ t (dry weight, dw) ha}^{-1}$ [3]. A 5% loss of material was subtracted from the field yield [53], and 93% of the switchgrass solids were VSS, which brings a yield of 8.84 t ha^{-1} of volatile solids. This would amount to a methane yield of $2600 \text{ m}^3 \text{ ha}^{-1}$ or $91.1 \text{ GJ (0.0346 GJ for each m}^3 \text{ of methane) of gross energy (Table 5)$, which is similar to what was measured for grass silage ($2700 \text{ m}^3 \text{ ha}^{-1}$) [48]. The energy used during the operation of an anaerobic digester fed with switchgrass (heating of digester, leaks, operation of digester, switchgrass production) was estimated to 13 GJ ha^{-1} [3]. The net yield from pretreated

Table 5 – Comparison of net energy yields from switchgrass under different conditions of harvest and treatment.

Assays	Methane produced ($\text{ml g}^{-1} \text{ VS added}$)	Methane yield ($\text{m}^3 \text{ ha}^{-1}$)	Net energy	
			(GJ ha^{-1}) ^a	(MWh ha^{-1}) ^b
WHS	140	1200	29.8	3.0
SHS	205	1800	49.7	5.0
SHS ^c	287–298	2600	78.1	7.6

WHS: winter harvested switchgrass. SHS: summer harvested switchgrass.

^a 13 GJ removed from the gross energy yield for growth and harvest of switchgrass, and operation of the digester.

^b Net electricity generated at 30% efficiency (2.88 kWh per $\text{m}^3 \text{ CH}_4$).

^c SHS with the best pre-treatment option (pectinases or Mn peroxidase coupled with alkalization).

switchgrass would then represent 78.1 GJ, although not taking into account the energy cost of the pre-treatment. This is higher than what was estimated by Martinez-Pérez et al. [54], from a theoretical scenario involving the production of hydrogen in a first acidification step, then methane production in a second stage, for a total of 69 GJ ha⁻¹. The conversion of methane into electricity at a 30% efficiency rate (2.88 kWh per m³ of methane), would generate a gross 7583 kWh, or net 6554 kWh from pretreated switchgrass.

The best methane production obtained from the different assays using mulched switchgrass without pre-treatments, 205 ml g⁻¹ VS, would generate a gross yield of 1800 m³ ha⁻¹ or 49.7 GJ, which is lower than the 61.2 GJ estimated by Samson et al. [3]. In the event it would not be possible to obtain ensiled switchgrass, much less energy would be recovered from winter harvested switchgrass. In effect, with a methane production of 140 ml g⁻¹ VS, using the same conversion factors as above, a net energy production of 29.8 GJ or 3.0 MWh of electricity would be obtained (Table 5). This is still more than twice as much methane as what could be recovered from similar crops such as straw of oat or rapeseed which yielded 400–600 m³ ha⁻¹ of methane [36], although less than ensiled grass (900–1900 m³ ha⁻¹) such as reported by Amon et al. [55].

Also, the net energy generated as methane compares well with the production of ethanol from switchgrass. In effect, reported ethanol yield from switchgrass or similar biomass vary between 280 l t⁻¹ (dw) [56] and 340 l t⁻¹ (dw) [57] which can be converted into 6.6–8.0 GJ t⁻¹. This would represent 63–76 GJ ha⁻¹, which remains lower than the energy recuperated from switchgrass biomethanization after the pectinases pre-treatment.

4. Conclusions

To our knowledge, this paper reports for the first time the methane yield from switchgrass. Although extensive work has been conducted in the field with crops grown in Europe, there was a need to assess the methane potential of North American native perennial grasses. The methane yield from winter harvested switchgrass remained low even when pre-treated and thus would not represent an interesting candidate for biomethane production. However, fresh harvested switchgrass showed promising methane yields, using soft physico-chemical and/or enzymatic pre-treatment. Therefore switchgrass represents an interesting candidate as a lignocellulosic crop for methane production. The best approach to have a constant supply of this feedstock would be ensiling. Switchgrass has not been ensiled yet to our knowledge, as it is not used for animal feeding. Future research could then focus on performing switchgrass ensiling and confirm the methane yield from that feedstock. There is also a need for the optimization of the pre-treatment. A maximal impact for the lowest energy and cost input must be obtained.

Enzymatic pre-treatments could offer an alternative to more commonly used energy intensive physico-chemical pre-treatment, although its cost-effectiveness remains to be confirmed and/or improved. In effect, it does not require the use of potentially dangerous chemical, does not imply intensive pre-treatment conditions (heat, pressure) and does not

require complex and or expensive pieces of equipment to process. Additional work needs to be performed in order to optimize the type of enzymes used and their dosage.

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