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Abstract
Miscanthus has been studied and used for several energy vectors production such as bioethanol. For anaerobic digestion it presents a low methane potential but this potential can be improved either by genotype selection or pretreatment. Eight different miscanthus genotypes belonging to *M. x giganteus*, *M. sacchariflorus* and *M. sinensis* species were studied. In a second time, alkali pretreatments (NaOH 10g $100g_{TS}^{-1}$, CaO 10g $100g_{TS}^{-1}$) were applied in different operational conditions : temperature, time, solids content, particle size on Flo genotype. The methane potential varied between miscanthus genotypes with values ranging from 166 ± 10 $NmL_{CH4}\ g_{VS}^{-1}$ to 202 ± 7 $NmL_{CH4}\ g_{VS}^{-1}$. Regarding the pretreatments and operational conditions tested in this study, soda is more efficient than the lime. All of the studied pretreatments increased the kinetics and the methane production (from 17% to 121%).

Keywords
Alkaline pretreatments; genotypes; miscanthus; anaerobic digestion

INTRODUCTION

The emerging biorefinery concept is attractive to optimize the energy-crop use. Miscanthus presents several advantages: genotype and phenotypic variability [2], few inputs requirements and a high aboveground biomass production [3]. Few studies have considered miscanthus as anaerobic digestion feedstock showing quite low methane potential. For example, Fryedendal-Nielsen and al.[5] reported around 120 $NL_{CH4}.kg^{-1}_{VS}$ for *Miscanthus x giganteus* harvested in February. Early harvest led to a 30% higher methane production but is not consistent with a several-year crop. Two strategies can be considered to improve the methane production. The first one is to select a genotype leading to high methane yield. Indeed the biochemical composition and thus the holocelluloses accessibility varied according to genotype [1]. The second one is to study biomass pretreatments especially the efficient ones on lignocellulosic biomasses. There are key steps in biorefinery for cell wall deconstruction and increase the accessibility of sugars to enzymes and microorganisms. Among efficient pre-treatments for delignification, chemicals and more precisely alkaline have been highlighted [6]. Soda pretreatment is the most studied, classical parameters are alkali dose 1-10 % ($g_{NaOH}\ g_{TS}^{-1}$), temperature around 40-60°C, duration from 0.5 to few days and solid loading from 30 to 100 $g.L^{-1}$ [4] [11]. However sodium presence can be detrimental for both anaerobic microorganisms or for soil quality if digestate is used as fertilizer. The use of another alkali agent such as lime which also presents the advantage of lower cost is favored, but the performance of both alkali should be compared.

We hypothesised that BMP (Biochemical Methane Potential) are impacted by genotype and can be improved by the use of chemical pretreatment. The aims of this study are (i) to compare the biochemical composition and methane yield of different species and genotypes of miscanthus and (ii) to study the impact of different alkaline pretreatments for one genotype, focusing on the use of different alkali (soda, lime). In addition, pretreatments conditions requiring low energy and water inputs were favoured in order to facilitate their industrial application.

MATERIALS AND METHODS

Miscanthus

Eight miscanthus genotypes were grown in North of France [1]: three belonged to *M. x giganteus* species (Floridulus, Gid and H8), four belonged to *M. sinensis* species (Goliath, Malepartus, Augustfeder and H6), and one belonged to *M. sacchariflorus* (H5). They were harvested in the eight year of cultivation (February 2015). Samples were dried for 4 days at 64°C and grounded to 1 mm, first with a crusher (Viking, model GE 220, France) at a coarse size and then ground with a hammer crusher (Gondard Productions model, France). Floridulus clone used for pretreatment study was crushed to around 4 cm with a knife mill (Retsch GmbH SM1).

Alkaline pretreatments

Soda (NaOH, Sigma) and lime (CaO, Akdolit® Q90; purity ≥ 92%) pretreatments were carried out in quatriplicate in 500 mL flask using 2 g_{TS} of miscanthus in conditions reported in Table 1. The alkali dose (10$g_{Reagent}$ 100g_{TS}^{-1}) was selected according preliminary experiments (data not shown). High TS (Total Solids) loading (200 g L^{-1}) were selected to test conditions with low water input and compared with classical low TS loading (40 g L^{-1}). Finally, experiments were carried out at room temperature and without mixing. Directly after pre-treatment, BMP tests were carried out in triplicate. Remaining pretreated samples were filtered through a 0.25-mm sieve to separate the solids from the liquid fraction for further chemical analysis.

Table 1: Pretreatments conditions (NaOH and CaO doses were at 10$g_{Reagent}$ 100g_{TS}^{-1} and all pretreatments were performed without mixing at room temperature)

Pretreatment	Particle size (mm)	Duration (h)	Dry matter content (%TS)
NaOH liquid	20	96	4
CaO liquid	20	96	4
NaOH 4d	1	96	20
NaOH 4d	20	96	20
NaOH 6d	1	144	20
NaOH 6d	20	144	20
CaO 4d	1	96	20
CaO 4d	20	96	20
CaO 6d	1	144	20
CaO 6d	20	144	20

Measure of methane potential

Classical BMP

Pretreated and controls (unpretreated) samples were digested in batch anaerobic flasks. The volume of each flask was 500 mL, with a working volume of 400 mL. The flask contained a bicarbonate buffer ($NaHCO_3$, 50 g L^{-1}), macroelements and oligoelements solutions whose compositions are given by (Monlau, et al., 2012) [7] and anaerobic sludge at 5 g_{VS} L^{-1} and the substrate at 5g_{TS} L^{-1}. Degasification with nitrogen was carried out to obtain anaerobic conditions. Triplicate bottles were incubated at 35°C during 60 days. Biogas volume was monitored by using a manometric device (LEO 2, KELLER). Biogas composition was determined as described in (Sambusiti et al. 2012) [9]. Methane production curves were modelled by a first order kinetics according the following equation where V is the volume of methane (NmL_{CH4} g_{VS}^{-1}), Vmax the maximum volume of methane which could be produced (NmL_{CH4} g_{VS}^{-1}), K the first order kinetic constant (d^{-1}) and t the digestion time (d).

$$V = Vmax(1 - e^{-Kt})$$

Automatic measure of methane potential

AMPTS (Automatic Methane Potential Test System) (Bioprocess Control AB, Lund, Sweden) was used for measuring the methane potential of each genotype. All of the samples were digested in batch 500 mL anaerobic flasks with a working volume of 400 mL. The flask were prepared with the same conditions as previously. Triplicate bottles were incubated at 35°C during 60 days.

Statistical analysis

Statistical analysis was performed using R software. The sources of variation of the biomethane potential was analysed using an ANOVA model.

RESULTS AND DISCUSSION

Impact of the genotype

The genotype had an impact on the organic matter ($p=1.1510^{-9}$), cellulose ($p=2.3410^{-8}$), hemicellulose ($p=1.9410^{-8}$) and Klason lignin ($p=3.3410^{-3}$) contents and consequently on their biomethane potential value ($p=1.0810^{-4}$) (Table2). The genotype H6 is the one with the highest BMP of 202 ± 7 NmL_{CH4} g_{VS}^{-1} (Table 2), it is the genotype with the lowest Klason lignin content. In contrast, the genotypes Flo and Gid had the lowest BMP (166 and 167 NmL_{CH4} g_{VS}^{-1} respectively) and the highest Klason lignin content even if it had a high cellulose content. It highlights the important role of the lignin as “a barrier” for the holocelluloses accessibility [8]. Flo genotype, which was one of the genotypes showing the highest lignin content and a lowest BMP value, was selected to study the enhancement of anaerobic digestion by alkaline pretreatments. In addition this genotype presents a high biomass yield [1].

Table 2. Dry mater content and biochemical composition and BMP (8^{th} year of cultivation).Values sharing a letter in common within a column do not differ significantly at p≤0.05

Species	Name	Organic matter (%TS)	Cellulose (%TS)	Hemicellulose (%TS)	Klason lignin (%TS)	BMP ($NmLg_{VS}^{-1}$)
M.x *giganteus*	M.floridulus	97.7± 0.02 ^{a}	38.6 ± 0.2 ^{a}	19 ± 0.7 ^{e}	25 ± 0.8 ^{a}	166 ± 10 ^{c}
M.x *giganteus*	Gid	97.8± 0.1 ^{a}	39.1 ± 0.2 ^{a}	19.9 ± 0.4 ^{d,e}	25 ± 2 ^{a}	167 ± 4 ^{c}
M.x *giganteus*	H8	95.1± 0.2 ^{d}	34.1± 0.2 ^{b}	22.7 ± 0.6 ^{b,c}	21 ± 2 ^{a,b}	201 ± 7 ^{a}
M.*sinensis*	Goliath	96.00±0.03^{c}	26.2± 0.2 ^{b}	22.3 ± 0.6 ^{c}	20 ± 2 $^{a, b}$	176 ± 11 ^{b,c}
M.*sinensis*	Malepartus	96.5± 0.01 ^{b}	34.5± 0.2 ^{b}	24.3 ± 0.4 $^{a b}$	22.4± 0.8 ^{a,b}	198 ± 4 ^{a,b}
M.*sinensis*	Augustfeder	96.3± 0.2 ^{b}	35 ± 1 ^{b}	24.6 ± 0.7 ^{a}	20 ± 3 $^{a, b}$	190± 12 ^{a,b,c}
M.*sinensis*	H6	94.0± 0.1 ^{e}	35 ± 1 ^{b}	22.4 ± 0.6 ^{c}	19 ± 2 ^{b}	202 ± 7 ^{a}
M.*sacchariflorus*	H5	98.00±0.04^{a}	37.8 ± 0.4 ^{a}	21.2 ± 0.5 ^{c,d}	25 ± 1 ^{a}	195 ± 8 ^{a,b}

Impact of the alkaline pretreatments

All of the alkaline pretreatments enhanced the BMP of at least 20% and the kinetics of minimum 17% (Table 3). The ones with soda with high solids contents are promising contrary to the “low solids content” ones (significantly different at 10%). Such a difference can be explained by lower initial pH in liquid pretreatments (12 versus 13). Statistical analysis was carried out for high solids content pretreatments where, each parameters (reagent, duration and particle size) were considered as a factor. The factor with the highest impact is the reagent ($p=1.0910^{-4}$), soda being more efficient than lime. The duration of the pretreatment and the particle size had no impact (p=0.079 and p= 0.286, respectively), as already shown by Sambusiti et al.[10] for the impact of sorghum biomass particle size (0.25mm to 2mm). The interactions between size and duration and between reagent and duration are not significant (p=0.40 and p=0.791, respectively). The interaction between reagent and size is significant at 10%. It seems that according the particle size, the reagent and not the duration of the pretreatment has an impact on the BMP. The improvement of the kinetics is better for the 20 mm than the 1 mm size. 1 mm substrate is more available for microorganisms than for the 20 mm one. Alkaline pretreatments are more useful for the 20 mm particle size which would be more used at farm biogas plants.

Table 3 : BMP, BMP and kinetics improvement for each alkaline pretreatments. Values sharing a letter in common within a column do not differ significantly at $p \leq 0.05$

Pretreatment	1 mm		20 mm	
	BMP $NmL_{CH4}\ g_{VS}^{-1}$ (*improvement %*)	*Kinetics improvement (%)*	BMP $NmL_{CH4}\ g_{VS}^{-1}$ (*improvement %*)	*Kinetics improvement (%)*
-	188 ± 14 [e] (-)	-	184 ± 15 [e] (-)	-
NaOH liquid 4d	-	-	228 ± 5 [c,d,e] (24)	54
CaO liquid 4 d	-	-	223 ± 17 [d,e] (21)	42
NaOH 4d	274 ± 11[a,b] *(45)*	*65*	257 ± 15 [a,b,c,d] (40)	121
NaOH 6d	291 ± 17 [a] *(55)*	*69*	269 ± 16 [a,b,c] (46)	98
CaO 4d	226 ± 11 [c,d,e] *(20)*	*17*	239 ± 6 [b,c,d] (29)	60
CaO 6d	245 ± 6 [b,c,d] *(30)*	*20*	223 ± 13 [b,c,d] (21)	69

CONCLUSION

Among the eight genotypes studied, BMP ranged from 166 ± 10 to 202 ± 7 $NmL_{CH4}\ g_{TS}^{-1}$. Genotype breeding could be interesting to produce miscanthus dedicated for methane production. Alkaline pretreatments with high solids contents are promising to improve the kinetics and the methane production. Soda showed best performances than lime but considering digestate use as fertiliser and detrimental impact of sodium on soils, lime pretreatment should be further optimized.

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