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Assessment of factors influencing the biomethane yield of maize silages

Frédéric Mayer [a,b], Patrick A. Gerin [b], Anaïs Noo [a], Guy Foucart [c], Jos Flammang [d], Sébastien Lemaigre [a], Georges Sinnaeve [e], Pierre Dardenne [e], Philippe Delfosse [a,*]

[a] Centre de Recherche Public – Gabriel Lippmann, Department "Environment and Agro-biotechnologies", 41, rue du Brill, L-4422 Belvaux, Luxembourg
[b] Université catholique de Louvain, Earth & Life Institute, Bioengineering, Croix du Sud 2, box L7.05.19, B-1348 Louvain-la-Neuve, Belgium
[c] Centre Indépendant de Promotion Fourragère, Croix du Sud 2, box L7.05.11, B-1348 Louvain-la-Neuve, Belgium
[d] Administration des Services Techniques de l'Agriculture, 16 route d'Esch, L-1019 Luxembourg, Luxembourg
[e] Centre Wallon de Recherches Agronomiques, 24 chaussée de Namur, B-5030 Gembloux, Belgium

HIGHLIGHTS

- Biomethane yield per hectare of maize silages was assessed.
- The cropping environment is the most influential factor for the biomethane yield per hectare.
- Late maturing maize varieties harvested at an early stage are advised for biomethanation.
- Volatile solids can predict the biochemical methane potential of maize silages.

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ABSTRACT

A large set of maize silage samples was produced to assess the major traits influencing the biomethane production of this crop. The biomass yield, the volatile solids contents and the biochemical methane potential (BMP) were measured to calculate the biomethane yield per hectare (average = 7266 m^3 ha^{-1}). The most influential factor controlling the biomethane yield was the cropping environment. The biomass yield had more impact than the anaerobic digestibility. Nevertheless, the anaerobic digestibility of maize silages was negatively affected by high VS content in mature maize. Late maturing maize varieties produced high biomass yield with high digestibility resulting in high biomethane yield per hectare. The BMP was predicted with good accuracy using solely the VS content.

1. Introduction

Providing sustainable solutions to meet the world energy demand is a key challenge for the 21st century (Advisory group on energy and climate change, 2010). Several strategies are considered but all scenarios investigated include the increase of renewable energy in the energy mix. The European Commission intends to achieve at least 55% of renewable energy in gross final energy consumption in 2050 (European Commission, 2011). In Luxembourg and Belgium, the target is to reach 11% and 13% respectively, of renewable energy in the gross final energy consumption by 2020 (European Parliament and Council, 2009).

Renewable energies mainly include solar energy (thermic and photovoltaic), wind power, hydroelectricity, geothermal energy and biomass. Local, easy-to-run and multipurpose solutions should be investigated among these various opportunities. Anaerobic digestion appears in this perspective to be a convenient and suitable solution because this biotechnology provides multiple answers to meet energy needs (heat, electricity and fuel), waste management and recycling, and fertilizer requirement for agriculture (Ward et al., 2008).

Anaerobic digestion, also known as biomethanation, is a bioprocess that involves microorganisms which convert organic material into biogas, under anaerobic conditions (Duncan and Nigel, 2003). The produced biogas is mainly composed of methane and carbon dioxide. It can be used in combined heat and power plants to produce both electricity injected in the grid, and heat for local needs (Doušková et al., 2010). More recently, the upgrading of biogas to biomethane allows the injection of the later into the gas grid (Ryckebosch et al., 2011).

One advantage of anaerobic digestion is that a wide variety of organic substrates can be used to produce energy (Weiland, 2009). The feedstock of an anaerobic digester can be liquid or solid materials and residues, originating mainly from food and feed

* Corresponding author. Tel.: +352 47 02 61 442; fax: +352 47 02 64.
E-mail address: delfosse@lippmann.lu (P. Delfosse).

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industries, agriculture or households. The amount and the composition of the produced biogas vary from one substrate to another. Anaerobic biogasification potential (ABP) and biochemical methane potential (BMP) assess the volume of, respectively, biogas and biomethane produced through anaerobic digestion, per unit of feedstock matter ($mL\,g^{-1}$) (Schievano et al., 2008). Various energy crops have been investigated for the purpose of biomethane production (Amon et al., 2007a). Among these, maize is the most commonly used crop for biogas production since it offers high crop yield, agricultural practices related to its cropping are well known, and maize varieties are available to fit most climatic conditions encountered around the world (Amon et al., 2007b; Poeschl et al., 2010).

For decades, plant breeders and farmers have assessed and improved the nutritive value of maize, either for feed or food. Nowadays, efforts are also made to improve maize biomethane yield per unit of cropped area, calculated according to the following equation:

$$\text{Biomethane yield } (m^3\ CH_4.ha^{-1}) = \text{BMP } (m^3\ CH_4.t^{-1}) * \text{biomass yield } (t.ha^{-1}) \quad (1)$$

To optimise the biomethane yield from maize, factors that influence both parameters, BMP and biomass yield, should therefore be identified and managed. Many factors such as the soil and weather conditions during cropping, the plant variety and the cultural practices used, strongly influence maize characteristics at harvest. These cropping factors influence both the composition and the production yield of the maize biomass. The biomass composition (water content and organic composition) then influence the ABP and the methane content in the biogas ($\%CH_4$) leading to various BMP values (Oslaj et al., 2010; Schittenhelm, 2008; Gao et al., 2012; Bauer et al., 2009; Vervaeren et al., 2010).

Eq. (1) used to calculate the biomethane yield can be further broken down following in Eq. (2):

$$\text{Biomethane yield} = (\%CH_4 * \text{ABP}) * (\text{VS} * \text{biomass yield}) \quad (2)$$

where $\%CH_4$ is the methane content in the biogas and VS is the volatile solids content of the biomass.

The present study focuses on the respective influence of $\%CH_4$, ABP, VS and the biomass yield on the biomethane yield of maize. For this purpose, various maize varieties were cropped in various environments and harvested at different dates to obtain a wide range of values of biomethane yields in the final dataset.

The aim of this study was first to assess the influence of the various factors on the biomethane yield, in order to identify the cropping parameters and strategies that can be used to optimise the energy production from maize through anaerobic digestion. A second aim was to determine a model to predict maize silage BMP from fast and easy-to-run experimental measurements.

2. Methods

2.1. Maize production and analytical measurements

In 2007, 2008 and 2009, maize was grown by the Administration des Services Techniques de l'Agriculture (ASTA) in Kehlen, Marnach, Nagem, Overpelt, Pletschterhof and Useldange in Luxembourg, and by the Centre Indépendant de Promotion Fourragère (CIPF) in Corroy-le-Grand, Perwez and Roux-Miroir in Belgium. More specifically, block design trials and randomised complete block design trials were carried out in 9 and 4 environments (field $\times$ year) respectively to produce variability in the harvested samples. Block design trials included a total of 25 different varieties from various seed companies and 1, 2, 3 or 4 field replicates. For all the maize varieties studied, the FAO maturity classes ranged from 220 to 340 except for the variety Peru, which has a maturity class of 900. Randomized complete block design trials focused on 4 varieties: Atletico (FAO-280), Lucatoni (FAO-340), Piazza (FAO-240), and Seiddi (FAO-300). For each of these four varieties, 12 (or 16 for Corroy-le-Grand in 2009) replication plots were cropped in order to harvest 4 field replicates at 3 different dates (4 dates for Corroy-le-Grand in 2009).

The wet weight (WW) biomass yield ($t_{WW}.ha^{-1}$) was measured for each sample at the time of harvest, with a mechanical harvester (Haldrup, Inotech Engineering GmBH, Germany). After harvest, the chopped biomass (particle size around 1–2 cm) was directly ensiled in sealed plastic bags and stored under vacuum at room temperature until laboratory analyses were carried out. The fermentation gas produced during the ensiling process was removed by opening the bag, packing the biomass and resealing the bag under vacuum. In general, this procedure had to be repeated twice to reach a stable ensiled sample. When several harvest dates were investigated, the first date was chosen to correspond to the targeted dry weight content of 25% relative to the wet weight (WW) for the maize crop and the following harvests were realised at one or two weeks intervals.

Total solid (TS) and volatile solid (VS) contents were quantified in the maize silages after 24 h drying in an oven at 105 °C, and after 6 h in a furnace at 550 °C, respectively.

2.2. ABP and BMP measurements

Biogas and biomethane productions were measured following the recommendations of the VDI 4630 standard (Verein Deutscher Ingenieure, 2006). The parameters related to the ABP and BMP assays are summarised in Table 2, as recommended by Raposo et al. (2011 and 2012). Each maize sample was analysed in triplicates. Anaerobic digesters consisted in 2L heavy-duty polypropylene bottles (Nalgene 2126-2000, Thermo Scientific) placed in water baths and kept at constant mesophilic temperature (37 °C). The lid of the digester was equipped with fittings (Nalgene 2162-0531, Thermo Scientific) and connected to a 10L gas-bag (Tecobag, Tesseraux Spezialverpackungen GnbH) through tubing (Tygon R-3603, Saint-Gobain). The digester lids and the venting port of the gas bags were rendered gas-tight using bi-component DP405 adhesive glue (3M Scotch-Weld, USA).

Each digester was filled with the inoculum and a maize sample at the start-up of the experiment. The inoculum was collected from a mesophilic anaerobic digester from the municipal wastewater treatment plant of Schifflange (SIVEC, Luxembourg). The inoculum was incubated at 37 °C for four days for exhaustion of the nutrients present in the inoculum and consequently to decrease the endogenous biogas production of the inoculum. Microorganisms in this inoculum face a wide variety of different organic matters contained in wastewater. This diversity is fully suitable and recommended for anaerobic digestion trials in the laboratory (Raposo et al., 2011). The precise amount of inoculum and maize were recorded at the time of filling the digester.

The produced biogas was measured on a daily basis during the first week, then once a week for the rest of the anaerobic digestion. It was quantified with a wet drum-type gasmeter (TG05 wet-type, Ritter). The biogas composition was analysed to determine the content (expressed as a volume percentage) in methane and carbon dioxide with specific infrared sensors (Dynament, UK). The gas volumes were normalised (273 K, 1013 hPa) according to the temperature and pressure conditions. Batches (triplicates) involving the inoculum alone and the inoculum fed with microcrystalline cellulose as a control substrate (Sigma–Aldrich) were carried out in parallel to the anaerobic digestion of maize samples in order to measure the biogas and biomethane volumes produced by the inoculum solely and to check the inoculum activity. At each gas measurement, averages of both biogas and biomethane productions inherent to the inoculum were subtracted from the biogas and biomethane volumes produced by the maize samples digested within the inoculum.

Cumulative biogas and biomethane productions were calculated at the end of the anaerobic digestion of maize samples to get ABP and BMP values. The ABP and BMP values were calculated with respect to the amount of wet matter added in the batch digesters (ABP_{WW} and BMP_{WW}), and then expressed per unit of volatile solids (ABP_{VS} and BMP_{VS}) using the VS content measured on another subsample. In total, 23 anaerobic digestion campaigns were conducted to analyse the 379 maize silage samples.

2.3. Statistical analysis

Each factor was summarised by descriptive statistics: number of samples (*N*), range from minimum and maximum values, mean and standard deviation (SD), kurtosis and skewness, and standard error of laboratory (SEL). Relative standard deviation (RSD) and relative standard error of laboratory (RSEL) were computed as the ratio between the SD or the SEL, respectively, and the mean. The ratio between SEL and SD (SEL/SD) was also computed for each factor. Standard deviations of ABP and BMP were calculated according to Miller and Miller (2010) to consider error propagation.

Statistical data analysis was carried out with SPSS, version 19 (SPSS Inc.). Normal distribution of a dataset was tested with the Shapiro–Wilk test. After assessing the normality of the sampling distribution, relationships between parameters were measured with the Spearman's correlation coefficient. Prior to any mean comparison, normality was verified as described previously and homoscedasticity was tested with Levene statistic.

For the RCBD trial, the effect of the environmental factor, the variety, and the harvest date was assessed on the biomethane yield with the generalised linear models (GLM) procedure. The effect size, which is a statistic that allows the quantification of the magnitude of the effect of one independent variable relatively to the others independent variables (Field, 2009), was calculated together within the GLM procedure.

Within each environment (field $\times$ year) presented, the biomethane yield, the $biomass_{VS}$ yield and the BMP_{VS} of each group, characterised by the variety and the harvest date or the variety solely, were compared. If normality and homoscedasticity of the sampling distribution were respected, analyses of variance (ANOVA) were carried out using the generalised linear models GLM procedure, followed by Tukey Post Hoc tests to compare means. The T3-Dunnet statistic was used in case of unequal variances for Post Hoc test. Kruskal–Wallis test was used if normality hypothesis was violated.

An α-risk of 0.05 was used as the significant probability level for all statistical tests.

Linear regressions and confidence intervals were calculated with SigmaPlot 12.5 (Systat Software, 2011).

3. Results and discussion

A set of 379 different maize samples was collected from the fields. This dataset is one of the largest sets investigated with the aim of testing biomethane production from maize (Raposo et al., 2012). Some batches were considered as invalid based on inadequate ABP and BMP productions by the standard substrate (microcrystalline cellulose) run simultaneously. For all the retained batches, the BMP_{VS} of the cellulose standard was on average 353 $mL.gVS^{-1}$ with a SD of 11 $mL.gVS^{-1}$ (Table 1), and similar to that generated in an interlaboratory study (Raposo et al., 2011). Descriptive statistics for biomethane yields, biomass yields, ABP and BMP were summarised in Table 2. The lack of biomass for four samples explains the lower number of samples ($N = 375$) for VS statistics. The invalid batches explains the lower number of samples ($N = 364$) available for ABP_{WW} and BMP_{WW} statistics. The combined missing data for VS on one hand, and ABP_{WW} and BMP_{WW} on the other hand, explain the lower number of samples ($N = 363$) for ABP_{VS} and BMP_{VS}. SEL, RSEL and SEL/SD were not computed for the biomethane yield, the biogas yield, the $biomass_{VS}$ yield and the $biomass_{WW}$ yield because there was only one measurement for the $biomass_{WW}$ yield.

3.1. Factors influencing the biomethane yield

Considering the whole dataset (Table 2), the average biomethane yield per hectare was 7266 $m^3\ ha^{-1}$, with a standard deviation of 1724 $m^3\ ha^{-1}$. The biomethane yield per hectare of maize silages was highly variable (RSD: 23.7%) in this dataset and similar to biomethane yield of comparable maize varieties reported in the literature (Schittenhelm, 2008; Oslaj et al., 2010; Amon et al., 2007b).

The progress of biomethane yield of four maize varieties over 3 or 4 harvest dates were studied in 4 different environments (Fig. 1). The cropping environment was characterised by the field location and the year, and included cropping factors such as the pedoclimatic situation, fertilizer scheme, and the crop rotation. The harvest date and the variety were analysed separately as specific factors influencing the biomethane yield.

When combining the different harvest dates and varieties, the average biomethane yields were 8642, 6539, 5846 and 4955 $m^3\ CH_4.ha^{-1}$, in Corroy-le-Grand 2009, Corroy-le-Grand 2008, Kehlen 2009, and Useldange 2009, respectively. The biomethane yield varied greatly among these four environments ($p < 0.001$). The effect size (Field, 2009) of the cropping environment was high ($r = 0.76$), indicating that this independent variable was the main cause for the variability in the biomethane yield per hectare, as compared to the variety and the harvest date. Consequently, the cropping environment was responsible for most of the variability of the biomethane yield per hectare in the crop trials and such diversity must be considered when assessing energy crops.

Significant differences ($p < 0.05$) were found for different varieties and different harvest dates within the environments of Kehlen 2009 and Corroy-le-Grand 2009, whereas the biomethane yields were not statistically different in Corroy-le-Grand 2008 and Useldange 2009 (Fig. 1). The biomethane yield per hectare was observed to decrease with later harvest dates in Corroy-le-Grand 2009. This indicates that yields are higher at early harvest dates.

The biomethane yield per hectare was also analysed in 3 environments where various maize varieties differing by their maturity class were cropped and harvested at a single date (Fig. 2). No significant difference between biomethane yields per hectare was found among the varieties within an environment.

Since the VS increase with later harvest dates in the different environments (Fig. 1), as already reported (Gao et al., 2012), the VS were used as a plant maturity indicator to sort maize silage samples of the entire dataset (Fig. 3).

While data relatively dispersed, a significant negative correlation ($r = -0.29$) in the correlation matrix (Table 3) and a negative slope coefficient in the linear regression (Fig. 3A) were observed between the biomethane yield and the VS content. From this relationship, it is concluded that mature maizes tended to produce less biomethane than immature ones, similarly to the trend observed in Corroy-le-Grand 2009. Early harvest of maize would allow producing more biomethane through anaerobic digestion, according to the data produced from this study.

As the biomethane yield per hectare is the result of the product of the BMP_{VS} with the $biomass_{VS}$ yield, the correlation coefficients

Table 1
Conditions used to perform the anaerobic biogasification potential (ABP) and the biochemical methane potential (BMP) assays.

Parameters	Value
Inocula	
Origin	MWTP (Schifflange, Luxembourg), mesophilic anaerobic digester
Number of batch campaigns	23
Total solids	2.2 ± 0.4 %WW
Volatile solids	1.2 ± 0.2 %WW
Activity	Checked with microcrystalline cellulose
Degassing period prior to assays	4 days at 37 °C
Control substrate	
Type	Microcrystalline cellulose
Total solids	96.2 %WW
Volatile solids	96.2 %WW
Amount and concentration at start-up of the experiment	10 gWW and 6 gVS.kg $Inoculum^{-1}$
ABP	706 ± 23 mL gVS^{-1}
BMP	353 ± 11 mL gVS^{-1}
Substrates	
Type	Maize silages
State	Wet
Total solids	31.7 ± 6.5 %WW
Volatile solids	30.4 ± 6.4 %WW
Amount (gWW) and concentration (gVS.kg $Inoculum^{-1}$) at start-up of the experiment	30.06 ± 1.7 gWW and 5.6 gVS.kg $Inoculum^{-1}$
Experimental conditions	
Replicates	3
Measurement system	Volumetric, drum-type gas meter
Type of gas analysed	Biogas
Biogas composition	Methane and carbon dioxide by specific infrared sensors
Operational conditions	
Reactor capacity	Total volume: 2 L, working volume: 1.6 L
Temperature	Mesophilic (37 °C), thermostatic water bath
Stirring	Manual, daily
Duration	No pre-incubation, 42–56 days
Headspace gas	No flushing at start-up
pH/alkalinity adjustment	No adjustment
Mineral medium	No mineral medium added
ISR	2.11 ± 0.93

MWTP: municipal wastewater treatment plant, TS: total solids, VS: volatile solids, WW: wet weight, ISR: inoculum to substrate ratio. Results are expressed as mean ± standard deviation for the various inocula, substrates tested and inoculum to substrate ratio (ISR).

Table 2
Descriptive statistics of measured and calculated parameters for the overall maize dataset. The $biomass_{WW}$ yield was measured at the harvest on the wet non-ensiled maize, whereas VS, ABP_{WW} and BMP_{WW} were measured on the wet maize silages. Other parameters were computed from the previous ones.

Statistic	Biomethane yield (m^3 ha^{-1})	Biogas yield (m^3 ha^{-1})	$Biomass_{VS}$ yield (tVS ha^{-1})	$Biomass_{WW}$ yield (tWW ha^{-1})	Methane content (%CH_4)	BMP_{VS} (mL gVS^{-1})	ABP_{VS} (mL gVS^{-1})	BMP_{WW} (mL gWW^{-1})	ABP_{WW} (mL gWW^{-1})	VS (%WW)
N	364	364	375	379	364	363	363	364	364	375
Minimum	2355	3843	5.9	26.0	51.6	276	472	39	68	14.2
Maximum	11,598	19,711	24.2	102.4	63.5	557	980	201	365	52.3
Range	9243	15,868	18.3	76.4	11.9	281	508	161	297	38.0
Mean	7266	12,863	17.3	59.8	56.3	418	743	126	225	30.3
SD	1724	2815	3.4	17.6	2.5	41	57	25	48	6.57
RSD (%)	23.7	21.9	19.7	29.5	4.4	9.9	7.7	19.8	21.3	21.7
SEL	–	–	–	–	1	22	40	5	10	0.90
RSEL (%)	–	–	–	–	1.79	5.3	4.42	3.97	4.44	2.9
SEL/SD (%)	–	–	–	–	50	54	60	20	21	14

WW: wet weight, VS: volatile solids, ABP: anaerobic biogasification potential, BMP: biochemical methane potential, N: number of samples, SD: standard deviation, SEL: standard error of laboratory, RSD: relative standard deviation, RSEL: relative standard error of laboratory.

between the different maize traits were determined (Table 3). The biomethane yield per hectare was highly and positively correlated with the $biomass_{VS}$ yield (r = 0.88), and less correlated with the BMP_{VS} (r = 0.65). The high correlation coefficient between the biomethane yield per hectare and the $biomass_{VS}$ yield (r = 0.88) led to a high coefficient of determination (R^2 = 0.84) of a first-order linear regression between these two factors (Fig. 4).

The RSD of BMP_{VS} (9.9%) was half the RSD of the $biomass_{VS}$ yield (19.7%). This indicates that the variability of anaerobic digestibility was lower than the variability of the $biomass_{VS}$ yield.

Most of the variability of the biomethane yield per hectare of maize silages can be explained by the variability of the $biomass_{VS}$ yield. Such an observation was already reported by German reports reviewed by Herrmann and Rath (2012), which found coefficients of determination of around 0.9 between the $biomass_{VS}$ yield and the biomethane yield. Both traits, $biomass_{VS}$ yield and BMP_{VS}, affected the biomethane yield per hectare with different weights. Factors influencing these two important traits were further investigated.

3.2. Factors influencing the $biomass_{VS}$ yield

One way of optimising the biomethane yield per hectare would be to increase the $biomass_{VS}$ yield. A high variability (RSD: 19.7%)

was found for the biomass$_{VS}$ yield in the dataset (Table 2), indicating the existence of opportunities to optimise and maximise this parameter. Indeed, the biomass$_{VS}$ yield is the result of the product of the biomass$_{WW}$ yield with the VS. The influence of these two parameters on the biomass$_{VS}$ yield was assessed.

High RSD values of 29.5% and 21.7% for the biomass$_{WW}$ yield and the VS (Table 2) respectively, offer large flexibility to alter both factors.

The correlation matrix (Table 3) shows a positive correlation ($r = 0.67$) between biomass$_{VS}$ yield and biomass$_{WW}$ yield, and a slight negative correlation ($r = -0.11$) between biomass$_{VS}$ yield and VS (also illustrated in Fig. 3C). There is also a negative correlation ($r = -0.77$) between the biomass$_{WW}$ yield and VS (Table 3 and Fig. 3D).

In most cases, the biomass$_{WW}$ yield decreased with late harvest dates (Fig. 1). The only exception was in Corroy-le-Grand 2008 where the biomass$_{WW}$ yield remained stable over harvest dates. Late maturing maize varieties tended to produce more biomass$_{WW}$ than early maturing ones.

In other environments (Fig. 2), late maturing maize varieties tended to yield more biomass$_{WW}$ with lower VS than early varieties. These trends are consistent because late maturing

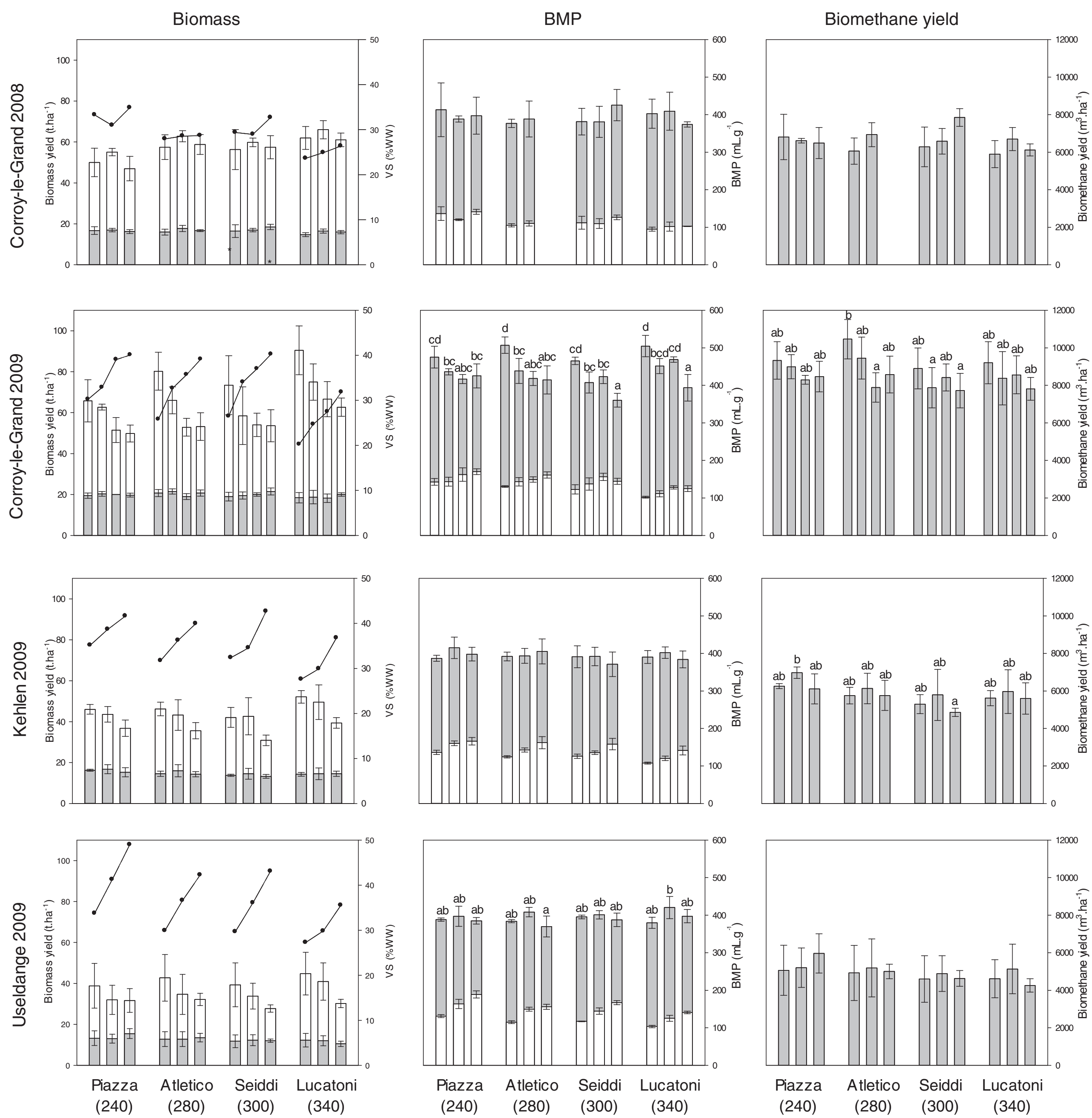

Fig. 1. Biomass yields (left), biochemical methane potentials (middle) and biomethane yields (right) of 4 maize varieties harvested at different harvest dates (3 or 4) in 4 distinct environments (rows). Biomass yields (biomass$_{WW}$ yield: white, biomass$_{VS}$ yield: grey) and BMPs (BMP$_{WW}$: white, BMP$_{VS}$: grey) are overlaid and not cumulated bars. VS content is represented by the line with black dots (left). For each variety, successive bars from left to right represent the three or four chronologically ordered harvest dates. FAO maturity classes are indicated in brackets. Error intervals represent the standard deviations. For the biomass$_{VS}$ yield, the BMP$_{VS}$ and the biomethane yield, bars holding different letters differ significantly ($p < 0.05$) within an environment.

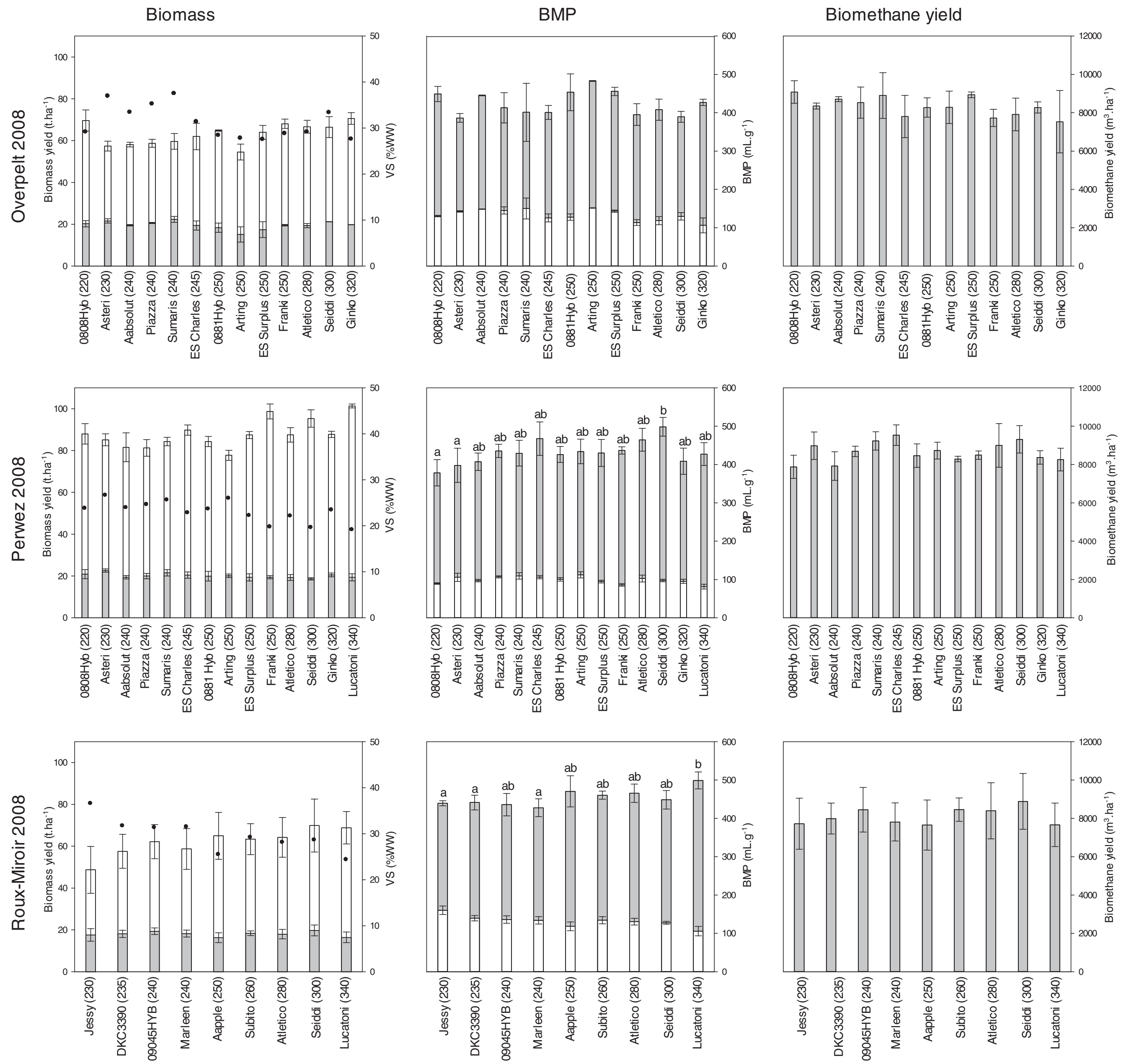

Fig. 2. Biomass yields (left), biochemical methane potentials (middle) and biomethane yields (right) of various maize varieties harvested in 3 distinct environments (rows). Biomass yields (biomass$_{WW}$ yield: white, biomass$_{VS}$ yield: grey) and BMPs (BMP$_{WW}$: white, BMP$_{VS}$: grey) are overlaid and not cumulated bars. VS content of the biomass is represented by black dots (left). FAO maturity classes are indicated in brackets. Error intervals represent the standard deviations. For the biomass$_{VS}$ yield, the BMP$_{VS}$ and the biomethane yield, bars holding different letters differ significantly ($p < 0.05$).

varieties need more time in the field to reach physiological maturity.

For all the groups (varieties × harvest dates) compared, the biomass$_{VS}$ yield did not differ significantly ($p < 0.05$) within an environment. The decrease of the biomass$_{WW}$ yield, balanced with the increase of VS, resulted in stable biomass$_{VS}$. This stability was assumed to be reached at an early maturity point not observed in these field trials.

According to these results, the best strategy to obtain the highest biomass$_{VS}$ yield is thus to focus on varieties that yield large amounts of wet biomass (late maturing varieties), and to delay the harvest until the biomass reaches a proper VS content that allows good quality silaging.

Maize VS content is an important parameter to consider in order to successfully obtain good quality silages. Too low VS content leads to losses of leachate with high contents of organic matter and soluble nutrients (Herrmann and Rath, 2012). In contrast, too high VS content prevents reaching a sufficient dense packing of the maize and proper anaerobic conditions, which leads to bad silage fermentation (Filya et al., 2006).

3.3. Factors influencing the BMP$_{VS}$

The BMP$_{VS}$ of maize silages were on average higher in this study (Table 2, mean: 418 mL gVS^{-1} and RSD: 9.9%) than BMP$_{VS}$ found in the literature (Plöchl et al., 2009; Bruni et al., 2010; Schittenhelm, 2008; Bauer et al., 2009), but the BMP_{VS} of cellulose run simultaneously were consistent as mentioned previously.

BMP ($mL_{CH4}\ gVS^{-1}$) is calculated from the ABP ($mL_{biogas}\ gVS^{-1}$) and the CH_4 content in the biogas (%CH_4). ABP_{VS} presents a low variability (Table 2, mean: 743 mL gVS^{-1}, RSD: 7.7%) and slightly decreases when VS increases (Fig. 3E). The methane content also slightly decreases when VS increases (Fig. 3B), with low variability around this trend (Table 2, RSD: 4.4%).The correlation matrix

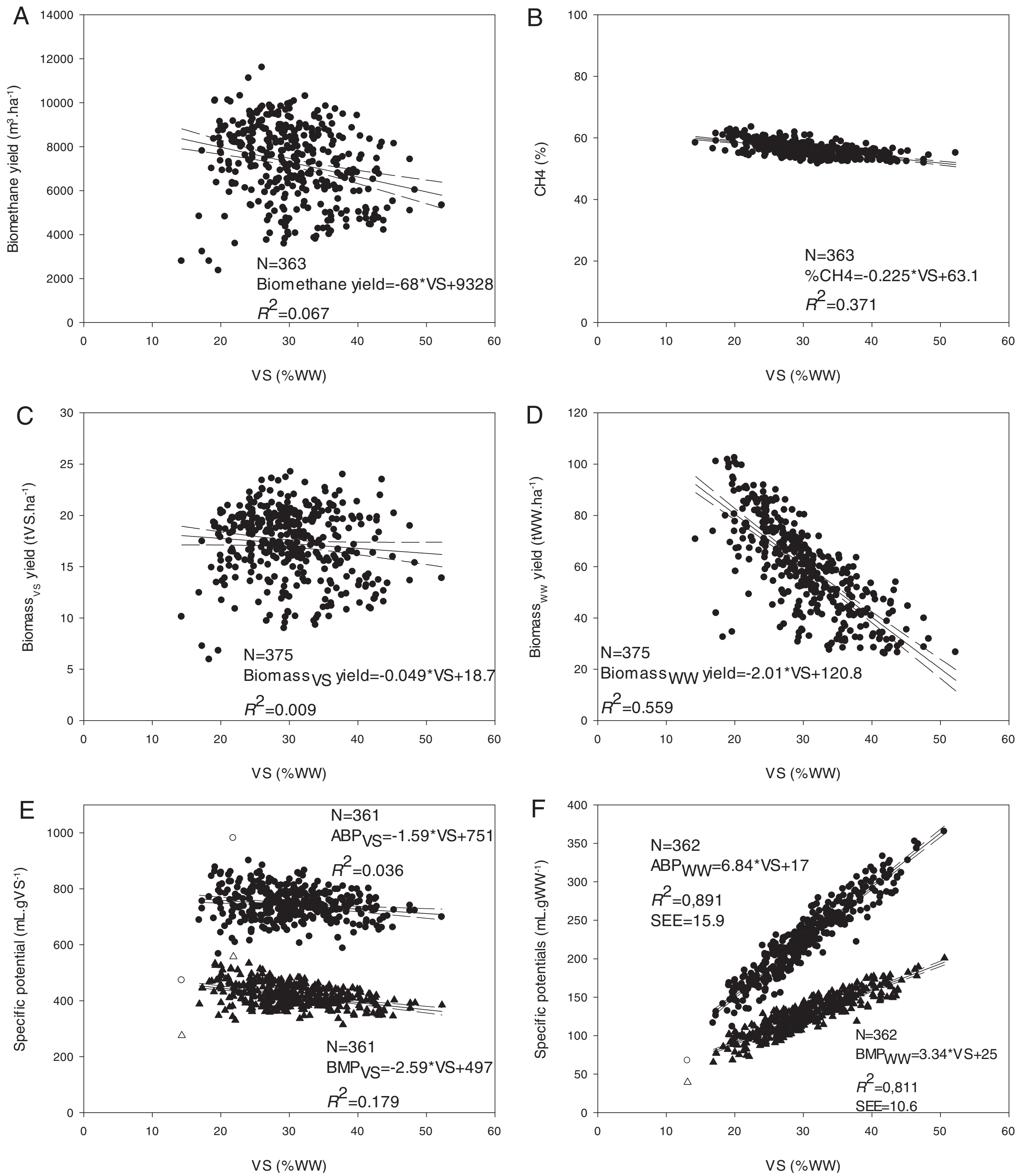

Fig. 3. Linear regressions between the volatile solid (VS) content and various maize traits analysed for biomethanation: (A) biomethane yield per hectare, (B) methane content in the biogas, (C) $biomass_{VS}$ yield, (D) $biomass_{WW}$ yield, (E) anaerobic biogasification potential (ABP_{VS}) or biochemical methane potential (BMP_{VS}) relative to volatile solids and (F) anaerobic biogasification potential (ABP_{WW}) or the biochemical methane potential (BMP_{WW}) relative to wet weigh (WW). Solid lines represent the linear regressions and dashed lines represent the 95% confidence bands. Closed symbols: samples included in the linear regression; Open symbols: outliers. *N*: number of samples included in the linear regression, R^2: coefficient of determination, SEE: Standard error of estimates.

Table 3
Spearman's correlation coefficients between biomethanation traits of maize.

Parameters	Biomethane yield (m^3 ha^{-1})	Biogas yield (m^3 ha^{-1})	Biomass$_{VS}$ yield (tVS ha^{-1})	Biomass$_{WW}$ yield (tWW ha^{-1})	Methane content (%CH_4)	BMP$_{VS}$ (mL gVS^{-1})	ABP$_{VS}$ (mL gVS^{-1})	BMP$_{WW}$ (mL gWW^{-1})	ABP$_{WW}$ (mL gWW^{-1})	VS (%WW)
Biomethane yield (m^3 ha^{-1})	1.00									
Biogas yield (m^3 ha^{-1})	0.98*	1.00								
Biomass$_{VS}$ yield (tVS ha^{-1})	0.88*	0.91*	1.00							
Biomass$_{WW}$ yield (tWW ha^{-1})	0.75*	0.68*	0.67*	1.00						
Methane content (%CH_4)	0.60*	0.46*	0.38*	0.67*	1.00					
BMP$_{VS}$ (mL gVS^{-1})	0.65*	0.57*	0.27*	0.46*	0.69*	1.00				
ABP$_{VS}$ (mL gVS^{-1})	0.49*	0.48*	0.12*	0.21*	0.32*	0.90*	1.00			
BMP$_{WW}$ (mL gWW^{-1}))	−0.02	0.06	−0.03	−0.63*	−0.34*	0.02	0.22*	1.00		
ABP$_{WW}$ (mL gWW^{-1})	−0.13*	−0.04	−0.10	−0.71*	−0.50*	−0.11*	0.14*	0.98*	1.00	
VS (%WW)	−0.29*	−0.19*	−0.11*	−0.77*	−0.60*	−0.38*	−0.16*	0.90*	0.95*	1.00

Sampling distributions are not normally distributed. WW: wet weight, VS: volatile solids, ABP: anaerobic biogasification potential, BMP: biochemical methane potential.
* ($p < 0.05$).

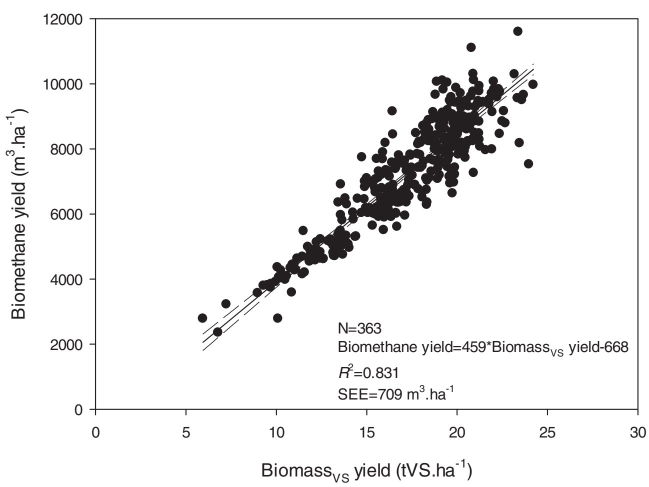

Fig. 4. Linear regressions between biomass yield and biomethane yield for the maize samples analysed. Solid lines represent the linear regressions and dashed lines represent the 95% confidence bands. N: number of samples, R^2: coefficient of determination. SEE: standard error of estimate.

(Table 3), shows a high correlation coefficient between ABP$_{VS}$ and BMP$_{VS}$ ($r = 0.90$).

The trends of ABP$_{VS}$ and %CH_4 to decrease for increasing VS content (Fig. 3E and B) explain the trend of BMP$_{VS}$ to decrease for increasing VS content (Fig. 3E).

Mature maizes with high VS content were characterised by lower BMP$_{VS}$, due to lower anaerobic digestibility and lower methane content, than maize silages with a lower VS content. Despite many factors related to the cropping conditions that could have affected the BMP$_{VS}$ of maize silages, the BMP$_{VS}$ distribution was not highly variable. The VS conversion into biomethane for maize silages showed lower flexibility as compared to the range wherein biomass yields can be achieved.

3.4. Characteristics of maize for biomethanation

Maize silages with lower VS tend to have slightly higher anaerobic digestibility (Fig. 3E), higher methane content in the biogas (Fig. 3B), and they produced high biomass yield in the field (Fig. 3D). Since the biomethane yield can be decomposed as the product of these factors (Eq. (2)), maize silages with low VS content were more favourable than mature maize for the biomethane production through anaerobic digestion.

Late varieties and an early harvest should then be investigated to improve biomethane production from maize silages. Such cropping practice could allow a high biomass production that could be left in the field until proper VS content for silaging is reached. Crop trials on maize with high maturity classes already reported good results (Schittenhelm, 2008; Oslaj et al., 2010). However, discussion and strategies about the best maize for anaerobic digestion are still ongoing (Herrmann and Rath, 2012).

The biomethane yield of maize could probably be further increased if two-phase anaerobic digestion is used to valorise the silage. Indeed, the recent work of Orozco et al. (2013) indicated that a pre-treatment under the form of a thermophilic hydrolysis prior to a mesophilic digestion caused an increase of 30% in the BMP$_{VS}$ of grass silage. If such improvement can be achieved for maize silage, the average biomethane yield could exceed 9000 m^3 ha^{-1} under the conditions prevailing in Luxembourg and Belgium.

3.5. Prediction of ABP and BMP

Since moisture contained in maize does not contribute to biomethane production, relations between ABP$_{WW}$ or BMP$_{WW}$ and the VS content were investigated (Fig. 3F). An outlier, corresponding to the sample with the lowest VS content, was excluded because of its singularity in the scatterplot. High Spearman's correlation coefficient are observed in the correlation matrix (Table 3) between VS and both ABP$_{WW}$ ($r = 0.95$) and BMP$_{WW}$ ($r = 0.90$). The VS content explains most of the variability observed for ABP$_{WW}$ ($R^2 = 0.89$) and BMP$_{WW}$ ($R^2 = 0.81$) (Fig. 3F). The ABP$_{WW}$ and BMP$_{WW}$ linearly increased with the VS content and could be modelled according to the equations in Fig. 3F. The first-order linear regression allows then to predict ABP$_{WW}$ and BMP$_{WW}$ from the VS. Precision of these simple models (SEE of 15.9 and 10.6 mL gWW^{-1} for ABP$_{WW}$ and BMP$_{WW}$ respectively) appears to be good as compared to the accuracy of the reference method (SEL = 10 mL gWW^{-1} and 5 mL gWW^{-1} for ABP$_{WW}$ and BMP$_{WW}$ respectively, as determined in batch anaerobic digestion). Using these equations as predicting models can be a useful tool when considering the time needed, 42–56 days (Table 1) to achieve a BMP batch assay. Such good results can be explained by the low variability in CH_4 content in the biogas and a low variability of the digestibility between the cropped maize varieties. Indeed, these maize varieties are the results of years of breeding efforts to optimise the yield and digestibility of maize used as animal feed. Such linear regressions and prediction equations could prove useful for defining quality criteria (determination of expected ranges for ABP$_{WW}$ and BMP$_{WW}$ on the basis of a simple VS measurement) when carrying out batch anaerobic digestion assays. However, ABP$_{VS}$ and BMP$_{VS}$ cannot be predicted on the basis of VS as input data (R^2 equal to 0.026 and 0.155 for ABP$_{VS}$ and BMP$_{VS}$ respectively).

3.6. Precision of measurements in batch assays

The SEL values, which characterise the repeatability of the method, were 10 and 5 mL gWW^{-1} for ABP_{WW} and BMP_{WW} respectively, and 40 and 22 mL gVS^{-1} for ABP_{VS} and BMP_{VS} respectively (Table 2). The RSEL was around 4–5% for ABP_{WW}, ABP_{VS}, BMP_{WW} and BMP_{VS}. While the RSD values, which characterise the dispersion within the population, were 21.3% and 19.8% for ABP_{WW} and BMP_{WW}, respectively, they dropped down to 7.7% and 9.9% for ABP_{VS} and BMP_{VS} respectively. The SEL and the SD of ABP_{VS} and BMP_{VS} were close to each other as indicated by the SEL/SD ratio of 60% for ABP_{VS} and 54% for BMP_{VS}, whereas the SEL/SD ratio was around 20% for ABP_{WW} and BMP_{WW} (Table 2).

For ABP_{VS} and BMP_{VS}, the average dispersion of the repeated measurements for one sample is higher than half the range of all observed values. Thus, the method used for estimating ABP_{VS} and BMP_{VS} is repeatable (low RSEL) but the accuracy is too low within the observed range of measurements to give the exact value of ABP_{VS} and BMP_{VS}.

Whereas the method presented here to measure the ABP_{WW} and the BMP_{WW} is fully suitable to assess the biomethane yield of maize silage, another method should be considered to accurately measure the ABP_{VS} and the BMP_{VS} of maize silages. A potential improvement for the accuracy of ABP_{VS} and BMP_{VS} measurement is envisaged through the analysis of maize silage as dried samples to avoid large interference due to high water content.

4. Conclusion

The main cause of variability of biomethane yield of maize silage was the cropping environment. The best advised maize for optimising anaerobic digestion is a late maturing variety harvested at an early stage to produce high biomass yield with low, but suitable for silaging, VS content. To further increase the biomethane yield of maize dedicated to biomethanation, improvement of the maize VS digestibility is suspected to be less rewarding than increasing the maize $biomass_{VS}$ yield per cropped area. BMP_{WW} was linked to VS content and first-order linear regressions allowed a quick prediction of both ABP_{WW} and BMP_{WW}.

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