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Biomethane yield of energy crops and prediction of their biochemical methane potential (BMP) with near infrared spectroscopy (NIRS)

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Doctoral thesis in
agronomic sciences and biological engineering

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Abstract

Biomethane yield of energy crops and prediction of their biochemical methane potential (BMP) with near infrared spectroscopy (NIRS)

Sustainable energy production is one of the major challenges for the future. Among existing energy production technologies, anaerobic digestion process produces biomethane as a source of renewable energy. The feedstock of such a process includes various biomasses and especially energy crops. To optimize the energy production, energy crops with a high biomethane yield per hectare should be identified. **The first objective of this work was to highlight the characteristics of energy crops that produce a high biomethane yield through anaerobic digestion.**

A large number (1147) of samples of maize (509 samples), tall fescue (426), sorghum (74), spelt (37), miscanthus (30), immature rye (28), switchgrass (27), sunflower (12) and hemp (4) was cropped. The fresh biomass yield per hectare was measured at the harvest and the biomass was then preserved as silage. The volatile solids (VS) content and the biochemical methane potential (BMP) were measured on crude wet silages. The BMP was the volume of biomethane that was produced per biomass mass unit ($mL_{CH4}.g^{-1}$) after 42 days of anaerobic digestion in a batch assay.

Considering the fresh biomass yield per hectare and the BMP of crude wet silages, maize (annual plant) was shown to be the best methane yielding plant per hectare. Green miscanthus (perennial plant) appeared as a promising alternative to maize thanks to methane yields similar to maize ($5.5 \pm 1\ 10^3\ m^3.ha^{-1}$and $5.3 \pm 1\ 10^3\ m^3.ha^{-1}$ respectively). The cropping of miscanthus and its biomethane production should now be assessed over its lifespan. For the various energy crops tested, the biomethane yield per hectare was significantly more influenced by the biomass yield per hectare, especially on a VS basis ($R^2 = 0.94$), than by the BMP.

The VS content of wet silages was the main factor controlling the BMP on a crude matter basis (BMP_{CM} in $mL_{CH4}.g_{CM}^{-1}$). The VS content can thus be used as an accurate predictor of the BMP_{CM} for the studied plant species ($R^2 = 0.80$). This is the

result of a homogenous anaerobic digestibility of the organic matter of tested biomasses. Nevertheless, biomasses with high fibre content, such as miscanthus, switchgrass or straw, were less rapidly digested than other biomasses with low lignin content, such as maize. Long digestion time would be needed to digest such lignocellulosic biomasses.

The BMP measurement is essential for the assessment of the biomethane yield of any substrate. However, it is a time-consuming assay (at least one month) and requires dedicated scientific instruments. **The second objective of this work was to assess whether the biochemical composition or near infrared spectroscopy can be used as a tool to quickly predict the BMP of energy crops.**

In a first approach, the influence of the biochemical composition of maize silages on the BMP was investigated. Indeed, the biomethane production that occurs during the anaerobic digestion is due to the degradation and conversion of the organic molecules of the substrate. The VS content appeared as the main parameter influencing the BMP_{CM} of the wet maize silages (R^2 = 0.81), as well as all other energy crops tested. For maize silages, the content in fibres, starch, proteins and fat was characterized more specifically. For the tested maize silages, the composition of the organic matter appeared to have no significant influence on the BMP_{CM} of the wet silages. However, when digesting dried and ground maize silages, the BMP_{CM} was influenced by the starch and fibres contents. Starch increased the BMP (R = 0.80), whereas fibres limited the anaerobic digestibility (R = -0.72 for cellulose and hemicellulose). The biochemical composition depends of the development stage of the plant at the harvest. Nevertheless the maturity, as characterized by the VS content, showed no clear effect on the BMP on a volatile solids basis (BMP_{VS} in $mL_{CH4}.g_{VS}^{-1}$).

In a second approach, the BMP was predicted with models based on NIRS. The NIR spectra of wet samples and of dried and ground samples were measured on silages of the harvested energy crops. Both spectra types were used as predictors of the BMP of crude wet silages. For maize silages specifically, models based on NIR spectra of wet silages were able to predict the BMP_{CM} with an accuracy similar to the one of the BMP assay, as shown by the standard error of prediction (SEP) of 7 $mL.g_{CM}^{-1}$ and the standard error of laboratory (SEL) of 5 $mL.g_{CM}^{-1}$. This can be explained (1) by the ability of NIRS to efficiently predict the VS content of wet silages

(SEP = 1%$_{CM}$) and (2) by the direct relationship between the VS content and the BMP_{CM}, as presented above. The accuracy of the prediction of the BMP_{CM} was lower when using a unique model for all plant species, as shown by the root mean square error of cross-validation (RMSECV) of 14 $mL.g_{CM}^{-1}$. Several prediction models specific to one plant species could be more accurate than a unique model.

The BMP_{VS} of wet silages was not well predicted according to the ratio of standard error of prediction to sample standard deviation for the cross-validation (RPDCV) of 1.6 and a RMSECV of 44 $mL.gVS^{-1}$. The BMP_{VS} range observed for all biomass samples was too limited, and the variability of the BMP measurement between replicates of the same sample was too large as compared to this BMP_{VS} range.

The repeatability of the BMP results was improved when digesting dry and ground maize silage samples instead of crude wet silages (SEL of 6 and 15 $mL.gVS^{-1}$, respectively). Moreover, the BMP_{CM} of such preprocessed substrates was accurately predicted with models based on NIRS (RMSECV of 7 $mL.gCM^{-1}$).

The NIR spectrum of the substrate (wet or dry and ground) used in the BMP assay is thus able to predict the BMP_{CM} of this substrate. NIRS is a non-destructive and fast analytical technology to estimate the anaerobic digestibility of biomass. Methods based on NIRS could facilitate and fasten the selection of energy crops dedicated to anaerobic digestion.

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Acronyms

2D: second derivative

ABP: anaerobic biogasification potential

ADF: acid detergent fibres

ADL: acid detergent lignin

BMP: biochemical methane potential

C: elemental carbon

CM: crude matter

CP: crude proteins

Det: detrend

FAO: food and agriculture organization

ISR: inoculum to substrate ratio

LV: latent variable

MC: mean centre

MWTP: municipal wastewater treatment plant

N: number of samples

NDF: neutral detergent fibres

NIRS: near infrared spectroscopy

PCA: principal component analysis

PLS: partial least squares

r: coefficient of correlation

R^2: coefficient of determination

Rdm: random

RMSE: root mean square error

RPD: ratio of standard error of prediction to sample standard deviation

RSD: relative standard deviation

RSEL: relative standard error of laboratory

SD: standard deviation

SEE: standard error of estimate

SEL: standard error of laboratory

SEP: standard error of prediction

SNV: standard normal variate

TS: total solids

VIP: variable importance in projection

VS: volatile solids

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Definitions

Anaerobic digestion: bioprocess also known as biomethanation that consists in the degradation and conversion of organic matter in methane by various microorganisms consortia in the absence of oxygen.

Bias: difference of the mean of the calibration set and the mean of the validation set.

Biomass: material made of and by living organisms and that results primarily from the photosynthetic capture of solar energy that is stored as chemical energy in organic materials. It includes wood and forests, agricultural productions, animals and aquatic resources from macro to microscopic scale, as well as their wastes.

Biochemical methane potential (BMP): volume of gaseous methane per unit of matter ($mL_{CH4}.g^{-1}$) produced during the anaerobic digestion of a substrate in a batch assay. The BMP can be expressed per unit of crude matter actually digested (BMP_{CM}) or per unit of volatile solids contained in the crude matter digested (BMP_{VS}). The BMP is usually measured after many weeks or months. Sometimes, it can be calculated at an infinite time from the experimental data with a curve fitting.

Biomethane yield: volume of biomethane produced by energy crops per hectare of cropped area ($m^3.ha^{-1}$). It is the product of the biomass yield ($t.ha^{-1}$) and the biochemical methane potential ($m^3.t^{-1}$).

Energy crops: plant species cropped in order to be valorised in energy (heat, electricity or fuel) through chemical, physical or biological process.

Near infrared spectroscopy: study of the interactions between matter and light in the range between 780 nm and 2526 nm (corresponding to 12820 to 3959 cm^{-1}). The absorbance spectrum is the figure representing the intensity of absorption by the matter versus wavelengths (or wavenumbers).

R^2_{max}: maximum coefficient of determination expected for a model. It gives clue about the feasibility of a prediction model.

Ratio of standard error of prediction to sample standard deviation for the calibration/cross-validation or prediction (RPDC/CV/P): statistics that give qualitative measure for the assemessment of the validation results. RPD between 2.5 and 3 indicates that the method is adequate for rough screening. RPD higher than 3 indicates that the method is adequate for screening, quality control (RPD > 5), or even excellent for analytical tasks (RPD > 8).

Root mean square error of calibration/cross-validation/prediction (RMSEC/CV/P): mean error of the concentration estimation in a model for the calibration, cross-validation or prediction.

Standard error of laboratory (SEL): mean error of the repeatability of the laboratory measurement.

Standard error of prediction (SEP): RMSEP corrected for the bias.

Variable importance in projection (VIP) scores: summary of the importance of a predictor for both predictors and predicted parameter. A variable with a VIP score close to or greater than 1 can be considered as important in a given model.

General introduction

Energy produced in a sustainable way is required for the future. Renewable energies are mostly investigated to fulfill such a requirement. Among various "green" technologies, the anaerobic digestion is considered as a valuable bioprocess that produces methane valorised in energy as heat, electricity or fuel. In biomethanation, the biomethane is produced from the degradation and conversion of organic matter. Among other substrates, plant species called energy crops are specifically harvested for energy production. However, according to the plant species or maturity at the harvest, energy crops don't yield the same amount of biomethane per unit of cropped area. Moreover, the estimation of the biomethane potential of energy crops involves a method that requires time (more than one month) and material. In this context, near infrared spectroscopy (NIRS), a fast and accurate analytical technologies which has already been successfully used to characterize biomass, could be use. Indeed, prediction models based on this tool could fasten the estimation of the biochemical methane potential (BMP).

The two major questions that are answered, through specific objectives, with the PhD thesis presented here are then:

- **What are the characteristics of energy crops with a high biomethane yield?**
  - What is the influence of the biomass yield on the biomethane yield?
  - What is the influence of the BMP on the biomethane yield?
- **Can the biochemical composition or near infrared spectroscopy be used in models that quickly predict the BMP of energy crops?**
  - What is the influence of the biochemical composition of the BMP of energy crops?
  - What is the ability of NIRS to predict the biochemical composition of energy crops?
  - Is the BMP predictable with models based on the biochemical composition or NIRS?

Chapter 1

Introduction

1. World energy situation

Since 1971, the total final energy consumption of the world increased (Figure 1.1, IEA, 2012a). In only 35 years, it has almost doubled from 4672 million tons of oil equivalent (Mtoe) in 1973 to 8677 Mtoe in 2010. This rise is due to the development of countries to satisfy people needs such as food production, health conditions, education, transport or entertaining activities. These different requirements call for different kinds of energy form: heat and electricity for households or fuel for transport for instance. To cope with these various needs, energy is produced from various resources and is rendered available under different vector types of fuel such as gas, liquids and solids.

Since the discovery of fire during Prehistory, wood was the main primary energy source until the 19th century. In 2010 (Figure 1.2), the main energy sources were oil, coal and peat, and natural gas. Sufficient and suitable energy production is a necessary condition to the safety of mankind. At the beginning of the 21st century, a wide choice of different technical solutions exists to respond to the world energy demand.

2. Conventional sources of energy

The three main energy sources (oil, coal/peat and natural gas) used since the 19th century are fossil fuels (Babeau, 2013). Fossil fuels are based on the extraction from the underground of different forms of hydrocarbons (liquid, solid or gas). Their combustion allows producing heat that may be later convert in a different form of energy (mainly mechanic or electric). These hydrocarbons are the result of the natural biodegradation of organic material million years ago. Nowadays, they form a stock of easily usable resource to produce energy. The vastness and availability of fossil fuel stocks were advantages to their use by mankind. The first and the second industrial revolutions were possible thank to coal and oil respectively. Since they have been developed for tens or even hundreds years, more than one technologies using fossil fuels are mature and profitable in the 21st century.

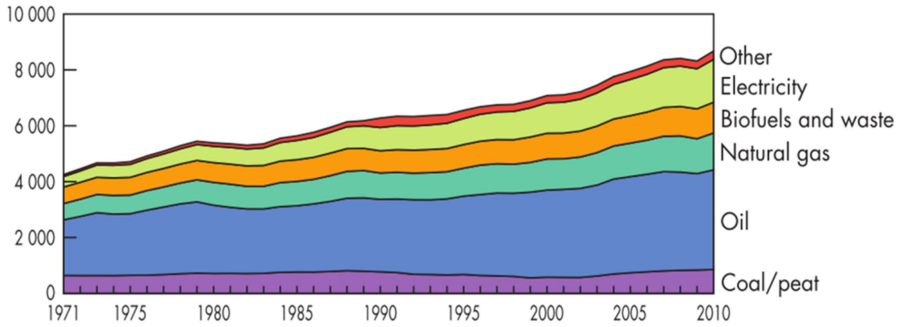

Figure 1.1. Progress of world total final consumption from 1971 to 2010 by fuel (Mtoe) (adapted from International Energy Agency, 2012a).

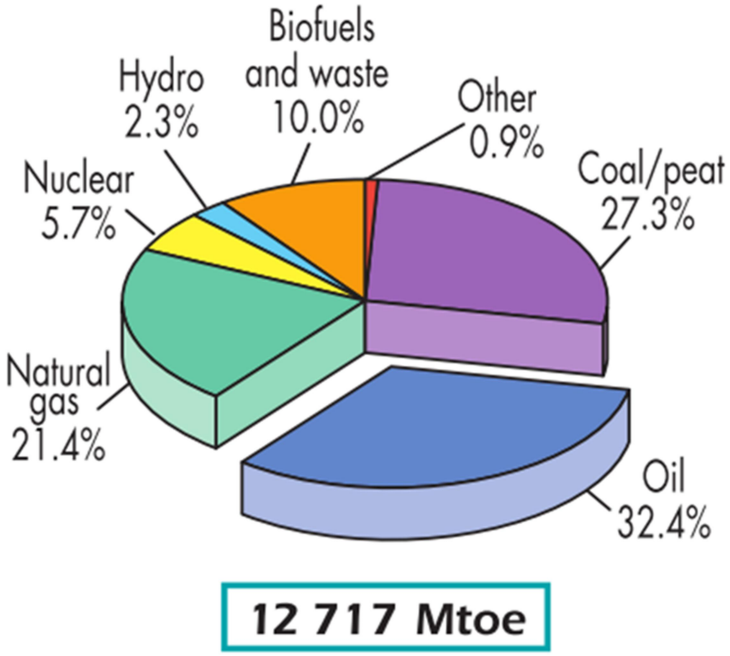

Figure 1.2. Fuel shares of world total primary energy supply in 2010 (International Energy Agency, 2012a).

Nevertheless the intensive use of fossil fuels shows nowadays their limits. The vast initial stock is partially consumed, the extraction of the remaining stock costs more and more and it is forecasted to stand for only several decades, up to one or two centuries according to the hypotheses that are used (The Colorado River Commission of Nevada, 2002). For instance, the current recoverable shale gas resource estimate provides enough natural gas to supply the United States for the next 41-82 years (Lior, 2012).

A second disadvantage of hydrocarbon-based energy is that carbon dioxide (CO_2) is released as a product during the combustion process. CO_2 is known to be a greenhouse gas (GHG). This gas can contribute to the global warming observed on Earth (Crowley, 2000). Since global warming on Earth is the cause of threats for humans and the economy (such as droughts or sea level increase), energy production sources and processes that do not emit additional CO_2 in the atmosphere must be investigated.

Nuclear power development appears as a way to avoid GHG emission. Nuclear industry is the fourth supplier of total energy (10.2%), after fossil fuels, in the OECD countries (data from 2011). Despite this relative low level at the world scale, some countries like France have highly developed nuclear energy so that it represents 75.5% of the electricity production in 2012 (EDF, 2012). Nuclear reactors use nuclear fission reaction to produce heat and then electricity. Nuclear reaction does not release any CO_2 during the nuclear fission since it is not based on hydrocarbon combustion. Less CO_2 is released in the atmosphere by the nuclear technology in its globality as compared to technologies based on fossil fuels. Nevertheless nuclear co-products and wastes are highly dangerous for living organisms because of their radioactivity. Recent accidents around the world (Three Mile Island, Tchernobyl, Fukushima) question the risk for health and environment of such an energy source (Butler, 2011).

Fossil fuels and nuclear power are efficient technologies to produce energy, but they have negative impacts on the environment. Both sources are not suitable for a sustainable development since they depend on limited stock of resources. Indeed, the generation of fossil fuels takes a long time at the human time-scale (thousands or millions of year), and nuclear fission is based on limited mineral availability. These resources are thus classified as non-renewable energies. On the contrary, renewable

energies that were already used for centuries before industrial revolutions can be part of the solution of a clean energy supply.

3. Renewable energies

Renewable energies include different technologies based on various resources (International Energy Agency, 2012b).

The sun is the main source of energy for Earth by providing light that is converted in heat and temperature gradients. Two technologies were developed to make use of the sun: the solar thermal energy to produce heat (International Energy Agency, 2012c) and the photovoltaic cells to produce electricity (International Energy Agency, 2010b). This resource is endless (on a human timescale) but inconstant due to weather conditions, time of the day and spatial situation.

Temperature gradients that are created by the solar energy are the source of wind power (International Energy Agency, 2013). This resource was used for thousand years with mills. Wind turbines can be installed anywhere there is wind, with little impact on the environment, even if the effect of high wind turbines on landscape is often questioned. Nevertheless, wind power is dependent of the occurrence of wind and its speed. Similarly to photovoltaic cells, the produced electricity cannot be efficiently stored.

Solar energy also feeds the water cycle. Hydroelectric power (International Energy Agency, 2012d) is generated through the displacement of water through different types of turbines. Use of natural or artificial water reservoirs allows producing energy when it is needed. However a hydroelectric reservoir can considerably modify the ecosystem through population displacement or through the upstream flood and downstream drying. Energy in water is also found in seas and oceans. Tides on the shore also create altitude difference that can be exploited similarly to hydroelectric dam. All of these facilities need specific location yet. In farther sea, marine streams are also used similarly to wind power, but offshore installations are expensive at the installation and for the maintenance.

Next to sun-derived sources of energy, another energy source is found in the soil and is defined as geothermal energy (International Energy Agency, 2010a). Geothermal energy uses the heat found in the earth's crust to generate electricity and provide heat. This resource is available in all regions, has no seasonal variation and no

impact on climate change. However, the initial costs to deploy this technology are high and there are low awareness and limited information about geothermal energy in general. All these energy technologies face limitations and barriers to their development. Availability of the resource and storage of the produced energy form are among the main limitations. Biomass is another resource derived from the sun energy. In the energy context, biomass appears as an alternative that can deal with some issues highlighted above.

4. Biomass as an energy source

4.1. Biomass resource

Biomass can be defined as the material made of and by living organisms (Damien, 2008). It results primarily from the photosynthetic capture of solar energy that is stored as chemical energy in organic materials (McKendry, 2002a; Nallathambi Gunaseelan, 1997). Biomass includes wood and forests, agricultural productions, animals and aquatic resources from macro to microscopic scale. Wastes from the mentioned resources are also included in the definition of biomass.

When considering biomass, energy is present within the chemical bonds of the structural components. Such a resource has thus the advantage to be storable and can be converted into energy when it is required.

The energy present in biomass is renewable because atoms that take part to chemical bonds are continuously recycled in various biogeochemical cycles, such as the carbon cycle, at a rate that is significant compared to human use. In this way, the CO_2 emitted from biomass was captured from the atmosphere in a time frame similar to human life. Carbon emitted as CO_2 through biomass conversion can then be considered as neutral for the environment (Demirbaş, 2001).

Well-managed production strategies are required when dealing with biomass for energy purpose. Biomass can require many years to be produced and used subsequently as an energy source. Some biomasses such as the agricultural productions are primarily dedicated to feed and food. Competition between energy production and people feeding may appear for the use of this resource and this dilemma must be faced with a sustainable approach (The High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security,

2013). Second generation biofuels derived from biomasses that have no food use, are now investigated. Their aim is to limit such a competition and to make a more sustainable use of the biomass production. However, fertile areas are still required to produce most of energy crops and sustaniable choices are essential for the use of such arable lands. The valorization of agricultural by-products and organic wastes must thus also be promoted to produce energy.

4.2. Conversion of biomass into energy

Biomass is among the most flexible energy resources. Indeed, it can be stored and converted into all energy vector forms (liquid, solid or gaseous fuel, heat and electricity). To achieve these conversions, two main process groups are used: thermochemical processes and biochemical processes (McKendry, 2002b; Bridgwater, 2003; Goyal et al., 2008). Mechanical processes also exist and consist mainly in milling and pressing oleaginous biomass mostly to recover oil (Figure 1.3).

The most common thermochemical process is the combustion. It is the oldest way of energy production and consists in the direct burning of biomass in the presence of oxygen (air). The produced heat must be directly used because long-term storage is not viable. Combustion can be implemented with all type of biomass, but it is feasible in practice only for biomass with low moisture content (<50-55% of the crude matter) or for biomass that has been dried (Jenkins et al., 1998).

Gasification process is a partial oxidation of biomass at high temperature (800-900°C). It converts biomass into a combustible gas mixture made of carbon monoxide, carbon dioxide, hydrogen and methane (syngas). The process appears as expensive compared to other technologies (Bridgwater, 2003).

Pyrolysis process is the thermal destruction of biomass in the absence of oxygen. It produces liquid oil, gases and solid products (char). Different process conditions lead to formation of products in different proportions (Goyal et al., 2008). This process is attractive because various forms of energy carriers (liquid, solid and gaseous) are produced and the products are of better quality compared to any other thermochemical process.

Among biochemical processes, alcoholic fermentation produces ethanol from materials that contain sugars, starch or cellulose. Carbohydrates are extracted from

the biomass and yeasts convert them into ethanol. In addition to carbohydrates, some biomass can also contain fat and oil (triglycerides). The best way to use this oil as fuel is to convert it into biodiesel through transesterification (Agarwal, 2007). During transesterification, triglycerides extracted from the biomass react with an alcohol to form esters and glycerol. Both liquid fuels, bioethanol and biodiesel, can be used in combustion engines. Nowadays, it is mostly used as a supplement for petrol in cars. However the primary resource to produce such energy carrier is limited to specific biomass that contains carbohydrates and/or fat. Moreover, the other important resources, such as nitrogen, phosphorus or potassium, contained in the process residues are often rendered non-available for recycling, in the vegetable production for instance.

Anaerobic digestion is another biochemical conversion technology that produces gaseous fuel and that is not limited to biomasses used for alcoholic fermentation or transesterification.

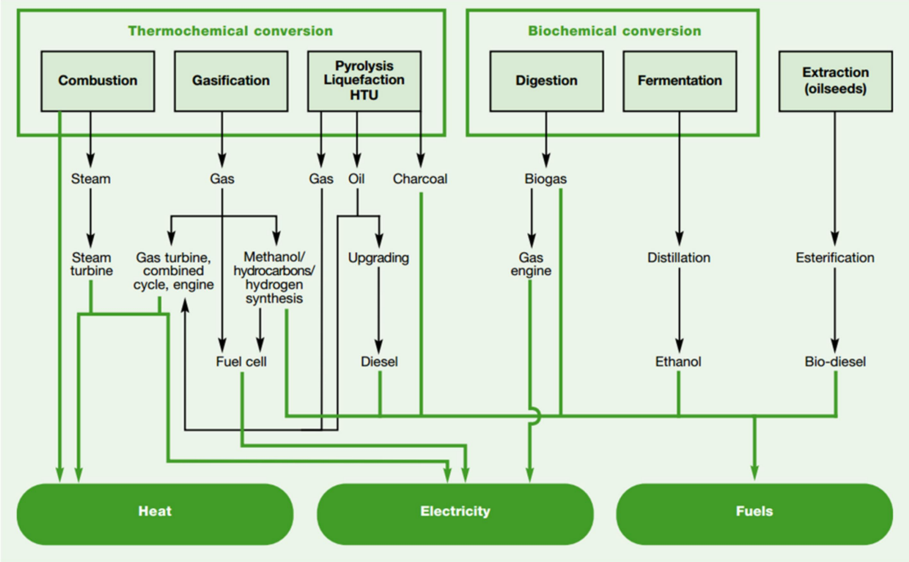

Figure 1.3. Main biomass energy conversion routes (United Nations Development Programme, 2000).

5. Biomethanation

5.1. The anaerobic digestion process

Anaerobic digestion is the biological degradation of organic substrates by microorganisms, achieved with no requirement for oxygen (Anderson et al., 2003). Such a biological degradation may be achieved without methane formation, but the term anaerobic digestion generally refers to the methanogenic process. Indeed, the energetic end-product of anaerobic digestion is methane. In addition to methane, the biogas produced during the anaerobic digestion contains a mixture of various gases, mainly carbon dioxide, but also hydrogen or hydrogen sulfide.

It is not a man-made industrial bioprocess and occurs naturally on Earth in watercourses, sediments or waterlogged soils (Ward et al., 2008). Methane is similarly produced in swamps where some glimmering lights can appear underneath the surface (Deublein & Steinhauser, 2008). Anaerobic digestion also occurs in the digestive tracts of animals like herbivorous mammals and wood-eating insects (Bayané & Guiot, 2010).

This biodegradation process can be exploited industrially in reactors (anaerobic digesters) as a way to produce a combustible gas from various biomass ressources. Methane production through anaerobic digestion is widely used for household needs around the world as a cooking fuel, especially in developing countries (Abbasi et al., 2012). In Europe, biomethane was first mostly produced as a co-product of the wastewater treatment process (Deublein & Steinhauser, 2008). Even if the methane production from agricultural wastes was known since Pasteur in 1884, and used for lighting major cities, this application was developed from the middle of the 20th century, consecutively to successive energy crisis. Biomethane plants can be installed in both agricultural places and in cities. As illustrated in Figure 1.4, methane can be valorized in heat and electricity in combined heat and power (CHP) systems (cogeneration). Purified methane can be also injected in the gas grid or used as a transportation biofuel. The residual fraction of the organic matter used in the process is called digestate and can be used in agriculture as a fertilizer and soil conditioner.

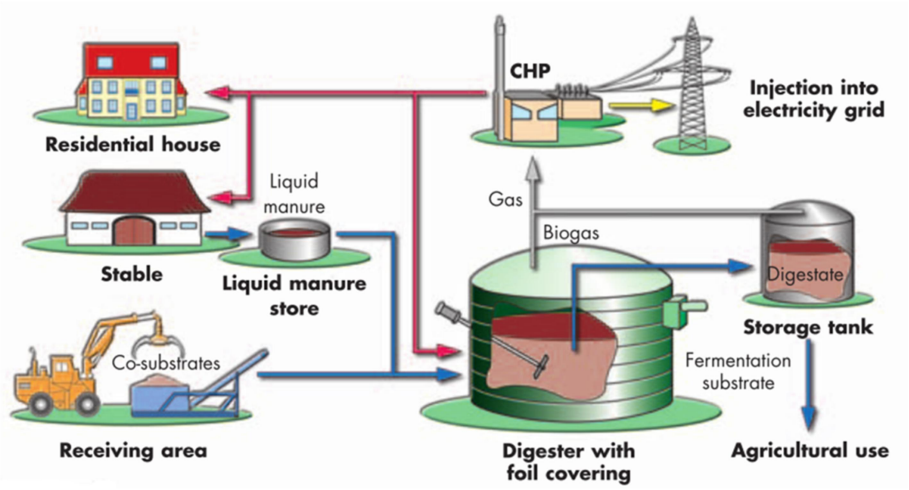

Figure 1.4. Scheme of processes in a farm-based biogas plant using co-substrates (Fachagentur Nachwachsende Rohstoffe, 2009).

When feeding an anaerobic digester with biomass, the substrate is involved in a very complex biological process that is commonly divided into four successive phases called the hydrolysis, the acidogenesis, the acetogenesis and the methanogenesis, as shown in Figure 1.5. During the first phase (hydrolysis), long and complex polymers like starch, cellulose, proteins and fats are dissociated into smaller molecules (short chain fatty acids, glycerol, peptides, amino acids, sugars). These monomers are then degraded into short-chain acids and alcohols during acidogenesis. Subsequently, the products of the acidogenesis are converted into acetate during acetogenesis. Carbon dioxide and hydrogen are also co-produced along the biochemical pathways of the acidogenesis and acetogenesis. Finally, the methane is formed through various chemical reactions described according to the molecules involved such as carbon dioxide and hydrogen (hydrogenotrophic path), acetate (acetotrophic path), or molecules with a methyl group (methylotrophic path).

Different groups of microorganisms act in each phase to degrade and convert the molecules. They have diverse optimal living conditions in terms of pH or tolerance to oxygen and various reactor designs have been created to optimize the process according these conditions. Indeed, the separation of the hydrolysis and acidogenesis phases from the acetogenesis and methanogenesis phases is already industrially realized, using baffled reactors or two-stage processes (Anderson et al., 2003).

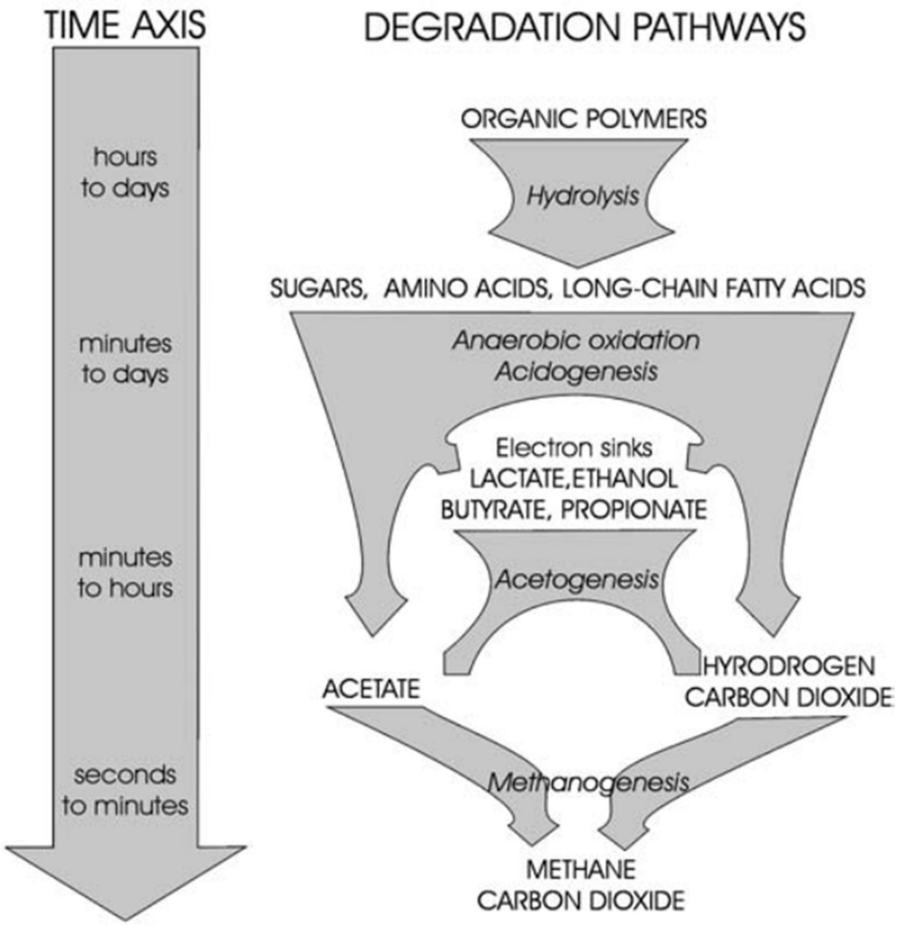

Figure 1.5. Degradation pathways in anaerobic digestion of organic matter. The parallel time axis corresponds to the conversion time of the organic matter through each degradation step (Ahring, 2003).

5.2. Advantages and disadvantages of biomethanation

The conversion of biomass through anaerobic digestion to produce biomethane offers various advantages for the environment and the economy (Anderson et al., 2003; Ward et al., 2008; Holm-Nielsen et al., 2009; Khalid et al., 2011).

Advantages

+ Biogas can substitute fossil fuels to produce a renewable energy through cogeneration of heat and electricity in combined heat and power units (CHP) (Pellerin et al., 1988). If upgraded to biomethane (Ryckebosch et al., 2011), it can be injected into the gas grid or used as a fuel for vehicles (Börjesson & Mattiasson, 2008).
+ The feedstock (biomass) and the product (biogas/biomethane) can be stored. The storage capacity allows some flexibility for the energy production according to requirements over time.
+ A wide variety of biomass can be used as a feedstock in anaerobic reactors: industrial and municipal wastewaters, sewage sludge, household and industrial wastes, farm residues and energy crops among others (Raposo et al., 2012).
+ The valorization of wastes reduces their impact on the environment.
+ In comparison to aerobic treatment, anaerobic digestion requires less energy and produced less biomass sludge (Low & Chase, 1999).
+ Pathogens and weed seeds are effectively reduced (Westerman & Gerowitt, 2013; Scaglia et al., 2014), especially for multi-stage digesters or with a pasteurization step included in the process. Many contaminants can be biotransformed (Stasinakis, 2012).
+ Odour emissions are reduced as compared to untreated manures (Hjorth et al., 2008; Ubeda et al., 2010).
+ Bioreactors prevent the release of methane to the atmoshpere from feedstock that would otherwise be stored in landfills or manure storage facilities that are known to emit GHG to the atmosphere. Meanwhile, burning the methane produces an energy that is carbon-neutral for the environment.

+ As compared to manure, the digestate is an improved fertilizer. The nutrients are saved in a geochemical cycle when spreading the digestate in agricultural fields (Al Seadi & Lukehurst, 2012). Synthetic fertilizers are less required, which decreases GHG emissions and fossil fuel consumption.
+ Anaerobic digestion is fully suitable with farming activities and represents an additional income if the technology is well managed (Hermes, 2007).

In this way, biomethanation is a technology that complies with many national strategies in term of energy production, air and water protection, waste management and fertilizer use as presented in Figure 1.6.

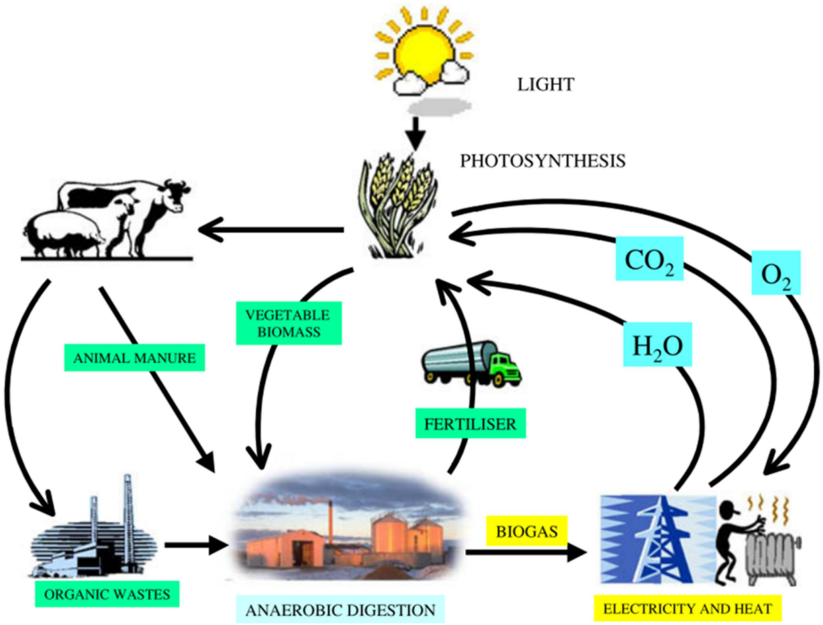

Figure 1.6. Schematic representation of the sustainable cycle of anaerobic co-digestion of animal manure and organic wastes (Holm-Nielsen et al., 2009).

Disadvantages

Stakeholders must also pay attention to some drawbacks caused by the biomethanation process.

- Some feedstocks need long retention time to be digested and pretreatments (alkali, thermal, thermochemical, ultrasonic, size reduction) or waste sorting appear necessary in some cases (Ward et al., 2008),.
- At the output of the process, the digestate quality must be checked, especially when treating animal products or wastes from unknown origin. When spread in agricultural fields, the digestate must respond to national regulations.
- Behind an apparent simplicity of the bioprocess, anaerobic digestion is sensitive to various physical, chemical and technical parameters. A process monitoring is required, where process analytical technology can be implemented (Holm-Nielsen & Esbensen, 2011).

5.3. Feedstock for anaerobic reactors

For biogas production, the chosen substrate is of prime importance because it has an influence on the biogas yield, it takes part to the biological reactions of the bioprocess and it conditions the status of the bioprocess (regular or imbalance). For instance, digestion of highly fermentescible matter like glucose produces quickly a high amount of intermediary compounds, especially volatile fatty acids, which inhibit the process (Marchaim & Krause, 1993). Digestion of proteins, urea and nucleic acids produces ammonia that may also inhibit the anaerobic digestion process if generated in too high quantities (Rajagopal et al., 2013).

For biomethanation, almost all types of biomass can be used as substrates as long as they contain carbohydrates, proteins, fats, cellulose and hemicellulose as main components (Deublein & Steinhauser, 2008). Biomasses with high lignin content are not suitable for anaerobic digestion. Residues from agricultural activities are particularly suitable for anaerobic digestion since a biomethane plant can be easily build up on a farm and digestate can be valorized on the farm land. Such residues include solid and liquid manure from various animals (ruminants, poultry, pigs, etc) and also crop residues. Agro-industrial residues are also commonly used.

Energy crops

Crops dedicated to energy production, called energy crops, are specifically produced for biomethanation. Farm-based biomethanation can take advantage of available agricultural machinery and practices to produce such a feedstock for anaerobic digesters. Energy crops represented 59% of the usable biogas potential in Germany in 2008 (Weiland, 2009).

The net energy yield per hectare of cropped area is the main parameter to consider when choosing energy crops. Many plant species and agricultural practices have thus been assessed to maximize this energy yield. One of the main parameters to consider is the biomethane yield per hectare. Indeed, it has been measured for plants from tropical locations (Nallathambi Gunaseelan, 2004; Nallathambi Gunaseelan, 2009a) up to the boreal zone (Seppälä et al., 2009; Pakarinen et al., 2011; Lehtomäki et al., 2008) and including temperate environments (Amon et al., 2007; Bauer et al., 2009).

Agronomic practices, such as the harvest date, or the harvest frequency for perennial crop, influence also the biomethane yield. The water need, the fertilizer requirement and the pest management must be considered since they have an effect on the production cost and the environmental benefits (European Environment Agency, 2006). Efforts are also engaged to crop novel energy plants on neglected or marginal lands in order to avoid the use of agricultural land primarily dedicated to feed and food production (Schröder et al., 2008).

In central Europe, maize is considered as the best energy crop for biomethanation because of its high biomass yield and a good conversion of its biomass to methane. Indeed, the biochemical composition of the biomass has an effect on the amount of produced methane. The effect of maize composition on the biomethane production has been widely studied. For maize, no consensus has been established so far about the influence of each biochemical fraction, such as proteins, lipids, fibres, soluble sugars or starch (Amon et al., 2007; Grieder et al., 2011; Rath et al., 2013).

The lignocellulosic fraction is important in energy crops. Such a fraction is recalcitrant to the anaerobic digestion process, especially because of the lignin content (Tsavkelova & Netrusov, 2012). Pretreatment have thus been investigated to enhance the degradation of lignocellulosic feedstock and its conversion in methane. Alkali pretreatment, thermal hydrolysis, ultrasonic break down, particle size

reduction or biological hydrolysis with cell lysate or specific enzymes have been assessed to increase the amount of methane produced by the substrate (Ward et al., 2008).

The highest amount of methane is thus searched for energy crops, as well as for other substrates. Indeed, the methane production of a feedstock is an important parameter to know to optimize biomethane production.

5.4. BMP measurement

The biochemical methane potential (BMP) is the amount of methane produced per unit of feedstock mass ($mL.g^{-1}_{substrate}$). It characterizes the methane quantity that a substrate can produce under anaerobic conditions and it is usually measured after several weeks (40-50 days) of anaerobic digestion (VDI, 2006).

The BMP is commonly measured in an anaerobic batch assay performed in small digesters (from 100 mL to several liters). The BMP batch assay consists in the incubation of a substrate with an anaerobic inoculum from an anaerobic digester and the measurement of the gas production over time, either from the pressure generated in the digester or directly from the gas volume released out of the digester. The degradation kinetic of the substrate can also be assessed when measuring the BMP to highlight an inhibition of the process owed to the sample or an inadequate inoculum for instance.

Behind an apparent simplicity of the method, many factors affect the BMP assay as highlighted in Table 1.1 (Raposo et al. 2011). The inoculum and the substrate are parameters that are difficult to control because of their biological origin. For instance, the amount of inoculum used to digest the sample, also known as the inoculum to substrate ratio, has been widely investigated and common values, which vary between 1 and 3 have been established to realize a BMP assay (Hashimoto, 1989; Raposo et al, 2006; Gonzalez-Fernandez & Garcia-Encina, 2009; Dechrugsa et al., 2013). The state of the substrate (crude and wet or dry and ground) can also have an influence on the BMP measurement (Ohl & Hartung, 2010). Furthermore, the scientific material can affect the gas estimation (Walker et al., 2009). In this context, a standardized protocol would be difficult to establish but efforts are undertaken in this way (Angelidaki et al., 2009). Moreover, such an assay is time-

consuming and requires dedicated equipments and manpower to carry out the fermentation and measure the gas production and composition (Hansen et al., 2004).

Table 1.1. Factors affecting the BMP assay (Raposo et al., 2011).

I. Inoculum
I.1. Origin
I.2. Characterization: pH, TS, VS, TSS, VSS
I.3 Amount (g) and concentration ($gVS.L^{-1}$) at start-up of the experiment
I.4 Activity
I.5 Time from sampling to starting test (days)
II. Substrate
II.1. Type part and particle size)
II.2. Characterization: moisture, TS, VS, TKN, organic fraction composition, atomic or elemental composition, fiber composition
II.3. Amount (g) and concentration ($gVS.L^{-1}$) at start-up of the experiment
III. Experimental conditionss
III.1. Quantification of gas
III.1.1. Measurement system (MS)
III.1.2. Type of gas
III.1.3. Biogas composition
III.2. Operational conditions
III.2.1. Physicals
Reactor capacity: working volume and total volume
Temperature: mesophilic/thermophilic
System: thermostatic water bath or chamber
Stirring: manual/automatic and continuous/batch
If automatic: magnetic bar/shaker If batch: times/day
Time: pre-incubation and test duration
III.2.2. Chemicals
Headspace gas
pH/alkalinity adjustement: if yes, chemical reagent and concentration at start-up of the experiment
Mineral medium: if yes, chemical composition and concentration at start-up of the experiment
III.2.2. Inoculum to substrate ratio

Other ways of estimating the BMP have been investigated to facilitate, fasten and compare results (Lesteur et al., 2010). The biochemical composition was the first indirect way investigated to estimate the BMP of a sample. Since the methane comes from the degradation and conversion of the biomass, models based on the biochemical composition have been assessed for decades to predict the BMP of various substrates (Buswell & Hatfield, 1936). The contents of cellulose, hemicellulose, lignin, fat, proteins, starch, soluble sugars or elementary composition were shown to be predictors of the BMP for various plant species (Amon et al.,

2007; Raju et al., 2011; Nallathambi Gunaseelan, 2007; Nallathambi Gunaseelan, 2009; Rath et al., 2013; Schievano et al, 2008; Schievano et al, 2009; Triolo et al., 2011). The determination of the biochemical composition from chemical methods is faster than the BMP assay. However biochemical analyses are still time-consuming and require different scientific equipments. Accessing rapidly the information on the suitability of an organic substrate for anaerobic digestion is a prerequisite to the further development of biogas production from organic wastes and energy crops. Therefore, a method fastening and facilitating the prediction of BMP would be highly valued by the biogas sector. In agriculture, the analysis of the biochemical composition of biomasses has been studied for decades. Fast and robust analytical techniques have been developed to characterize agricultural products. Among them, near infrared spectroscopy (NIRS) has been proved to be of particular interest.

6. Near infrared spectroscopy

The near infrared spectroscopy exploits the interactions between matter and electromagnetic radiations with wavelengths in the near infrared (NIR) range. Electromagnetic radiations (Figure 1.7) can be split in different regions according to the wavelength (in nm) or to the wavenumber (in cm^{-1}). The range that is visible by the human eye is called the visible spectrum of light and extends from about 360 to 780 nm (corresponding to 27778 to 12821 cm^{-1}). The ultra-violet is before 360 nm (or above 27778 cm^{-1}) and the infrared region is after 780 nm (or below 12821 cm^{-1}). The American Society of Testing and Materials (ASTM) defines the near infrared as the region between 780 nm and 2526 nm (or 12820 to 3959 cm^{-1}).

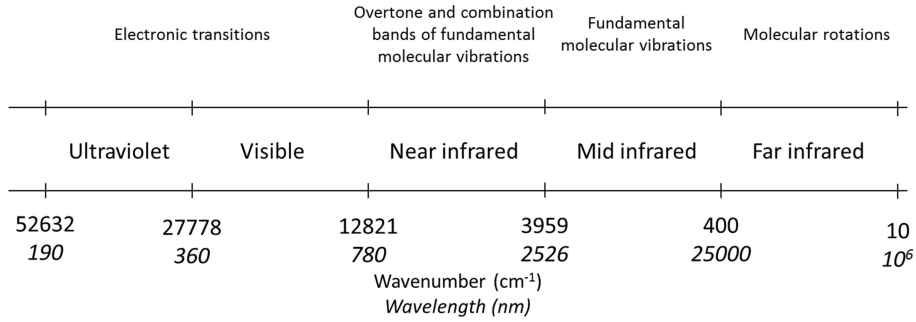

Figure 1.7. Spectroscpic regions of interest for chemical analysis (adapted from Burns & Ciurczak, 2008).

Matter is made of various atoms that constitute together molecules. Within molecules, atoms are linked with chemical bonds. According to the molecules and atoms, the chemical bonds can vibrate at different levels of energy. They can absorb energy and change from energy level if the exact energy is transmitted to them. Photons are considered as energy carrier with a specific wavenumber. Thus, the energy transmitted by a photon of exactly the right wavenumber can be absorbed by a molecule and excite the molecule bond from one energy level to another (Pasquini, 2003).

Energy levels of molecule bonds are definite and not continuous. The transition between the basal level and the first energy level corresponds to the fundamental vibration. The majority of absorption bands of covalent bond vibrations corresponding to fundamental vibrations is in the mid infrared (MIR) region between 3959 and 400 cm^{-1}. The vibrations are called overtones when the energy transition is realized through more than one level. Combinations are observed when interactions of different vibrational transitions occur (Burns & Ciurczak, 2007).

In the NIR region, most absorption bands are related to overtones and combinations of fundamental vibrations of -CH, -NH, -OH and -SH functional groups. According to the composition of the matter, the absorption will change. The figure representing the intensity of absorption by the matter versus wavelengths (or wavenumbers) is the absorption spectrum of a sample (Pasquini, 2003), as represented in Figure 1.8.

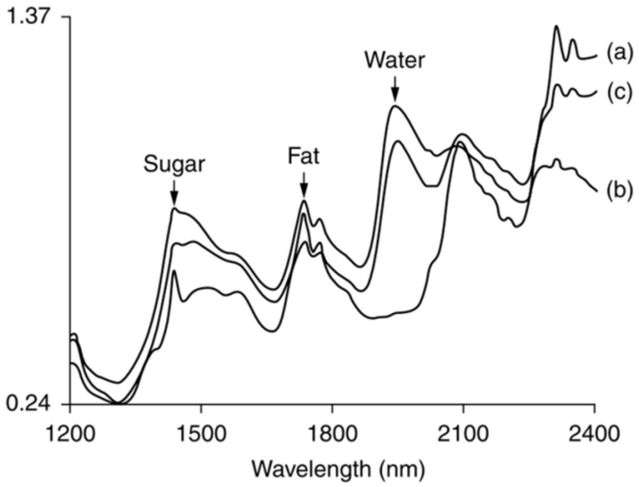

Figure 1.8. NIR spectra of mixtures of sugar/fat (a), sugar/fat/water (b), and sugar/fat/flour/water (c) (Burns & Ciurczak, 2007).

Typical NIR absorption bands are broad, overlapping and weaker than their corresponding fundamental MIR absorption bands. These characteristics require data processing of NIR spectra to relate spectral information to the sample chemical composition properties (Reich, 2005).

Chemometrics and multivariate analysis have been developed and used concomitantly to NIRS to extract information from spectral data (Naes et al., 2004). The most common analysis is the principal component analysis (PCA) used for qualitative purpose mainly. For quantitative purpose, multiple linear regression (MLR), principal component regression (PCR) and partial least square regression (PLSR) have been mostly used (Davies & Fearn, 2010). Other non-linear regression methods such as artificial neural network (ANN), locally weighted regression (LWR) or support vector machine (SVM) have been developed to improve the accuracy of models (Pérez-Marín et al., 2007).

NIRS coupled to chemometrics is a fast, non-destructive and multipurpose technology to analyse matter, and biomass especially. It requires minimum sample preparation and is suitable for on-line implementation in the industry. With such advantages and if complemented with chemometrics, NIRS appears as a powerful tool to predict the BMP as compared to the batch digestion assay, which is time-consuming and destructive. Indeed, the prediction of the BMP with models based on NIRS has been already investigated on organic wastes and some energy crops (Lesteur et al., 2011; Grieder et al., 2011; Raju et al., 2011; Doublet et al., 2011; Kandel et al., 2013). However, it was never investigated on wet maize silages or various energy crops silages.

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Chapter 2

Plan of the Thesis

Objectives and approaches

Energy crops are extensively investigated to produce biomethane as a source of renewable energy and a fast and convenient method to measure their biomethane productions is required instead of the time-consuming BMP assay.
In this context, the questions addressed by the present PhD work are:

- **What are the characteristics of energy crops with a high biomethane yield?**
- **Can the biochemical composition or near infrared spectroscopy be used in models that quickly predict the BMP of energy crops?**

To answer to these two major questions, the following specific questions will be addressed in the next chapters across the thesis:

Chapter 3*: *Assessment of factors influencing the biomethane yield of maize silages.

Maize is the preferred energy crops for biomethanation and is influenced by various agronomic factors from the seeding until the harvest, and also during preservation by the silaging process.

- **What is the influence of factors such as the biomass yield or the BMP on the biomethane yield of maize silages?**

The influence of the cropping environment, the harvest date, the maize variety, as well as the volatile solid (VS) content, was assessed on the biomethane yield.

Chapter 4*: *Biochemical composition of maize silages and its prediction with models using near infrared spectroscopy.

NIRS is widely used to characterize the biochemical composition of maize silages.

- **What is the biochemical composition of maize silages used in anaerobic digestion and how models based on NIRS are able to predict this biochemical composition?**

The biochemical composition of maize silages previously produced was characterized and the ability of models based on NIRS to predict the biochemical composition was assessed using spectra from wet biomass or from dry and ground biomass.

***Chapter 5*: Prediction of the biochemical methane potential of wet maize silages with biochemical composition or near infrared spectroscopy.**

The biochemical composition or the near infrared spectra are measured faster than the BMP on maize silages.

- **What is the ability of models based on the biochemical composition or NIRS to predict the BMP of wet maize silages?**

Biochemical and spectral data were investigated to create models that predict the BMP of wet maize silages, which were measured with the BMP batch assay after 40-50 days.

***Chapter 6*: Influence of sample preprocessing on the biochemical methane potential of maize silages and its prediction.**

The BMP were measured on crude wet matter, however dried and ground biomass is commonly used as a substrate for the BMP batch assay.

- **What is the influence of drying and grinding wet maize silages on the BMP and its prediction with biochemical or spectral data?**

The BMP of wet crude maize silages and dried and ground maize silage powders were measured after 56 days and compared. The prediction of the BMP of dry maize silage powders was assessed with models using the biochemical composition or NIRS as predictors.

While the previous chapters aimed at describing for maize silage specifically what are the main characteristics that influence its biomethane yield and if the biochemical composition or NIRS can be used to predict its BMP, the following chapters (Chapter 7 and 8) enlarge the study to other energy crops that have the potential to enter the cropping systems practiced in the Greater Region.

***Chapter 7*: Assessment of energy crops alternative to maize for biogas production in the Greater Region.**

Plant species other than maize can be cropped to produce energy through biomethanation.

- **What is the influence of the plant species on the biomethane yield per hectare?**

The biomethane yield of various plant species that can be alternative to maize for biomethane production were measured and assessed. Factors that influence the biomethane yield of these plant species were identified.

***Chapter 8*: Fast prediction of the biochemical methane potential of energy crops with near infrared spectroscopy.**

Models based on the VS content or NIRS can quickly predict the BMP of maize silages.

- **What is the ability of models based on NIRS to predict the BMP of various wet energy crops silages?**

The VS content and NIR spectra of wet silages and dried and ground silages were used in models to predict the BMP of various energy crops, and not only maize.

Chapter 3

Assessment of factors influencing the biomethane yield of maize silages

Adapted from:

Mayer F., Gerin P. A., Noo A., Foucart G., Flammang J., Lemaigre S., Sinnaeve G., Dardenne P. & Delfosse P. (2014). Assessment of factors influencing the biomethane yield of maize silages. *Bioresource Technology*, 153, 260-268. doi:10.1016/j.biortech.2013.11.081.

Biomethanation is seen as an environmental friendly process that produces biomethane as renewable energy. Energy crops are often co-digested in complement to manure to feed agricultural anaerobic digesters. Maize is among the preferred energy crops chosen for biomethanation. **What is the influence of factors such as the biomass yield and the BMP on the biomethane yield of maize silages?**

In the present chapter (Chapter 3), the biomass yield per hectare, the BMP and the biomethane yield per hectare of maize silages, cropped in Luxembourg and in Belgium, were assessed. Factors that influence these parameters were also investigated to find the best maize silages dedicated to biomethanation.

Abstract

A large set of maize silage samples was produced to assess the major traits influencing the biomethane production of this energy crop. The biomass yield, the volatile solid (VS) contents and the biochemical methane potential (BMP) were measured to calculate the biomethane yield per hectare (average = 7266 $m^3_{CH4}.ha^{-1}$). The most influential factor controlling the biomethane yield was the cropping environment. The biomass yield had more impact than the anaerobic digestibility because of its higher variability. Nevertheless, the anaerobic digestibility of maize silages was negatively affected by the high VS content of mature maizes. Late maturing maize varieties produced high biomass yields with high digestibilities resulting in high biomethane yields per hectare. The BMP was predicted with good accuracy using solely the VS content.

Keywords: biochemical methane potential (BMP), biomethanation, anaerobic digestion, biogas, maize silage, renewable energy, biodigestibility.

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 (Mara & Horan, 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 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 Equation 1:

$$\underset{(m^3_{CH4}.ha^{-1})}{\text{Biomethane yield}} = \underset{(m^3_{CH4}.t^{-1})}{\text{BMP}} * \underset{(t.ha^{-1})}{\text{biomass yield}}$$

To optimize 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 (%CH4) leading to various BMP values (Oslaj et al., 2010; Schittenhelm, 2008; Gao et al., 2012; Bauer et al., 2009; Vervaeren et al., 2010).

Equation 1 used to calculate the biomethane yield can be further broken down following in Equation 2:

$$\underset{(m^3_{CH4}.ha^{-1})}{\text{Biomethane yield}} = \underset{(mL_{CH4}.\ mL_{biogas}^{-1})}{\%CH4} * \underset{(m^3_{biogas}.tCM^{-1})}{ABP} * \underset{(t_{CM}.ha^{-1})}{\text{biomass yield}} * \underset{(g_{VS}.g_{CM}^{-1})}{VS}$$

where VS is the volatile solid content of the biomass and CM means crude matter.

The present study focuses on the respective influence of %CH4, 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 optimize 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. Material and 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 the *Centre Indépendant de Promotion Fourragère* (CIPF) in Corroy-le-Grand, Perwez and Roux-Miroir in Belgium. More specifically, block design trials and randomized complete block design trials were carried out in 9 and 4 environments (field x 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 focused on 4 varieties (Atletico, Seiddi, Lucatoni, Piazza) with maturity classes of 240, 280, 300, and 340 respectively. For each of the four varieties, 12 (or 16 for Corroy-le-Grand in 2009) replications plots were cropped in order to harvest 4 field replicates at 3 different dates (4 dates for Corroy-le-Grand in 2009).

The crude matter (CM) biomass yield ($t_{CM}.ha^{-1}$) 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 1-2cm) 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% on a crude matter basis for the maize crop and the following harvests were realized at one or two weeks intervals.

Total solid (TS) and volatile solid (VS) contents were quantified in the maize silages after 24h drying in an oven at 105°C, and after 6h 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 3.1, as recommended by Raposo (2011, 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). 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 crude matter added in the batch digesters (ABP_{CM} and BMP_{CM}), 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.

1.1. 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., 2005). 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.

Table 3.1. 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 %CM
Volatile solids	1.2 ± 0.2 %CM
Activity	Checked with microcrystalline cellulose
Degassing period prior to assays	4 days at 37°C
Control substrate	
Type	Microcrystalline cellulose
Total solids	96.2 %CM
Volatile solids	96.2 %CM
Amount and concentration at start-up of the experiment	10 gCM 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 %CM
Volatile solids	30.4 ± 6.4 %CM
Amount (gCM) and concentration (gVS.kg $Inoculum^{-1}$) at start-up of the experiment	30.06 ± 1.7 gCM 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 to 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

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

For the RCBD trial, the effect of the environmental factor, the variety and the harvest date was assessed on the biomethane yield with the generalized linear models (GLM) prodcedure. 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 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 generalized linear models GLM procedure, followed by Tukey post-hoc test 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).

2. 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 3.1), and similar to that generated in an interlaboratory study (Raposo et al., 2011). Descriptive statistics for biomethane yields, biomass yields, ABP and BMP were summarized in Table 3.2. 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_{CM} and BMP_{CM} statistics. The combined missing data for VS on one hand, and ABP_{CM} and BMP_{CM} on the other hand, explain the lower number of samples (N = 363) for ABP_{VS} and BMP_{VS}.

2.1. Factors influencing the biomethane yield

Considering the whole dataset (Table 3.2), 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 (Figure 3.1). The cropping environment was characterized 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 (Figure 3.1). 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 (Figure 3.2). No significant difference between biomethane yields per hectare was found among the varieties within an environment.

Table 3.2. Descriptive statistics of measured and calculated parameters for the overall maize dataset. The $biomass_{CM}$ yield was measured at the harvest on the wet non-ensiled maize, whereas VS, ABP_{CM} and BMP_{CM} were measured on the wet maize silages. The SEL of the various yields were computed from field replicates and not laboratory replicates. Other parameters were computed from the previous ones.

Statistic	N	Min	Max	Range	Mean	SD	RSD (%)	SEL	RSEL (%)	SEL/SD (%)
Biomethane yield ($m^3.ha^{-1}$)	364	2355	11598	9243	7266	1724	23.7	867	11.9	50.3
Biogas yield ($m^3.ha^{-1}$)	364	3843	19711	15868	12863	2815	21.9	1515	11.8	53.8
$Biomass_{VS}$ yield ($tVS.ha^{-1}$)	375	5.9	24.2	18.3	17.3	3.4	19.7	1.8	10.4	52.9
$Biomass_{CM}$ yield ($tCM.ha^{-1}$)	379	26.0	102.4	76.4	59.8	17.6	29.5	6.5	10.9	36.9
Methane content ($\%CH_4$)	364	51.6	63.5	11.9	56.3	2.5	4.4	1	1.8	50
BMP_{VS} ($mL.gVS^{-1}$)	363	276	557	281	418	41	9.9	22	5.3	54
ABP_{VS} ($mL.gVS^{-1}$)	363	472	980	508	743	57	7.7	40	4.4	60
BMP_{CM} ($mL.gCM^{-1}$)	364	39	201	161	126	25	19.8	5	4.0	20
ABP_{CM} ($mL.gCM^{-1}$)	364	68	365	297	225	48	21.3	10	4.4	21
VS (%CM)	375	14.2	52.3	38.0	30.3	6.57	21.7	0.90	2.9	14

CM: crude matter, 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.

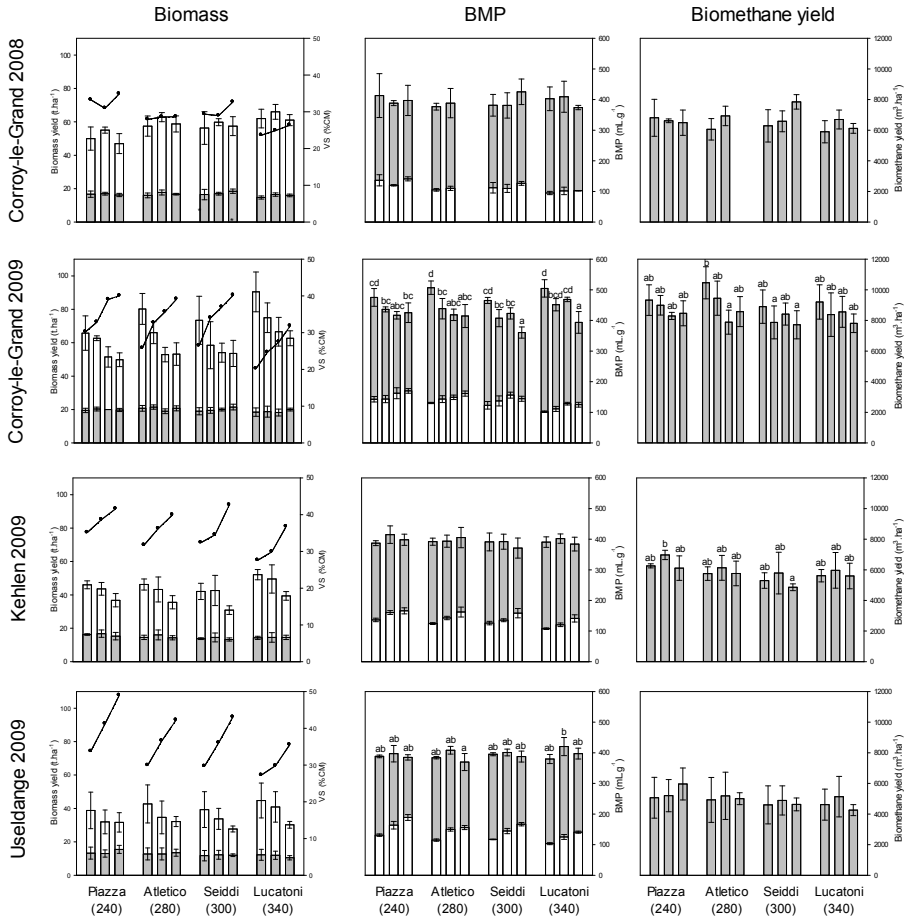

Figure 3.1. Biomass yields (left), biochemical methane potentials (middle) and biomethane yields (right) of 4 maize varieties harvested at different harvest dates (3 or 4) in 4 distinct environments (rows). Biomass yields ($biomass_{CM}$ yield: white, biomassVS yield: grey) and BMP (BMP_{CM}: white, BMP_{VS}: grey) are overlaid bars and not cumulated bars. VS content is represented by the line with black dots. 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. Uncertainty intervals represent the standard deviations. For the $biomass_{VS}$ yield, the BMP_{VS} and the biomethane yield, bars holding different letters differ significantly ($p < 0.05$).

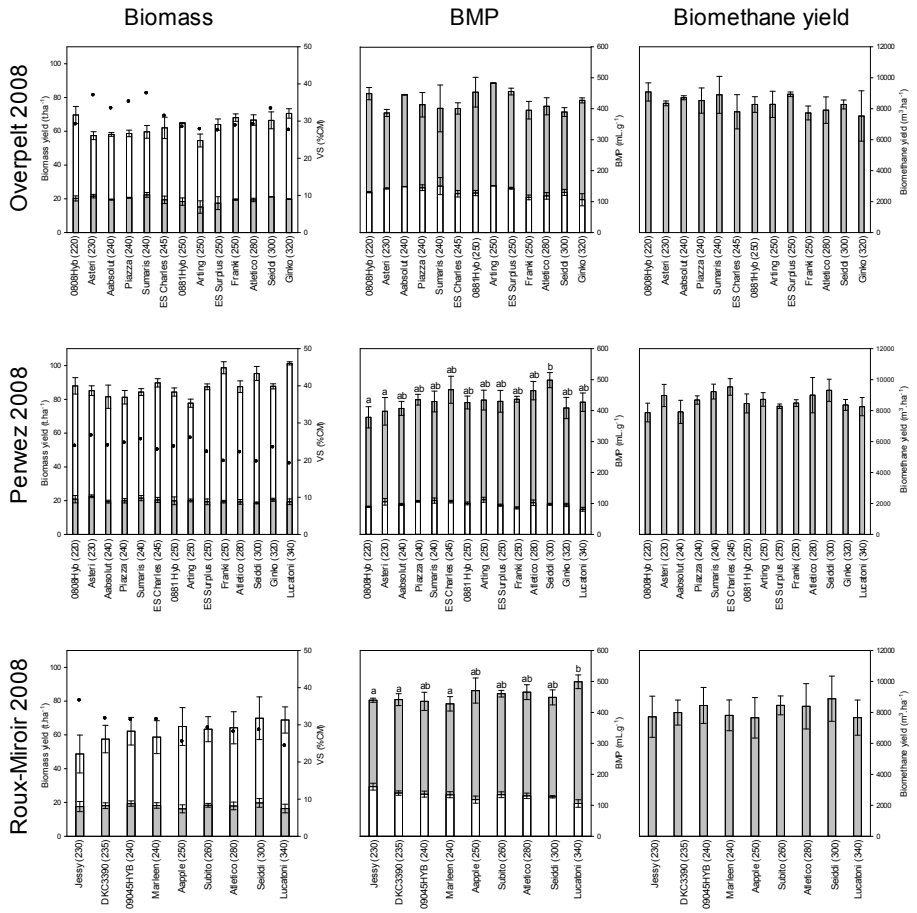

Figure 3.2. Biomass yields (left), biochemical methane potentials (middle) and biomethane yields (right) of various maize varieties harvested in 3 distinct environments (rows). Biomass yields ($biomass_{CM}$ yield: white, $biomass_{VS}$ yield: grey) and BMP (BMP_{CM}: white, BMP_{VS}: grey) are overlaid bars and not cumulated bars. VS content is represented by the black dots. FAO maturity classes are indicated in brackets. Uncertainty intervals represent the standard deviations. For the $biomass_{VS}$ yield, the BMP_{VS} and the biomethane yield, bars holding different letters differ significantly ($p < 0.05$).

Since the VS content increases with later harvest dates in the different environments (Figure 3.1), as already reported (Gao et al., 2012), the VS content was used as a plant maturity indicator to sort maize silage samples of the entire dataset (Figure 3.3).

While data relatively dispersed, a significant negative correlation (r = -0.29) in the correlation matrix (Table 3.3) and a negative slope coefficient in the linear regression (Figure 3.3A) 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 between the different maize traits were determined (Table 3.3). 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 = 0.83) of a first-order linear regression between these two factors (Figure 3.4).

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.

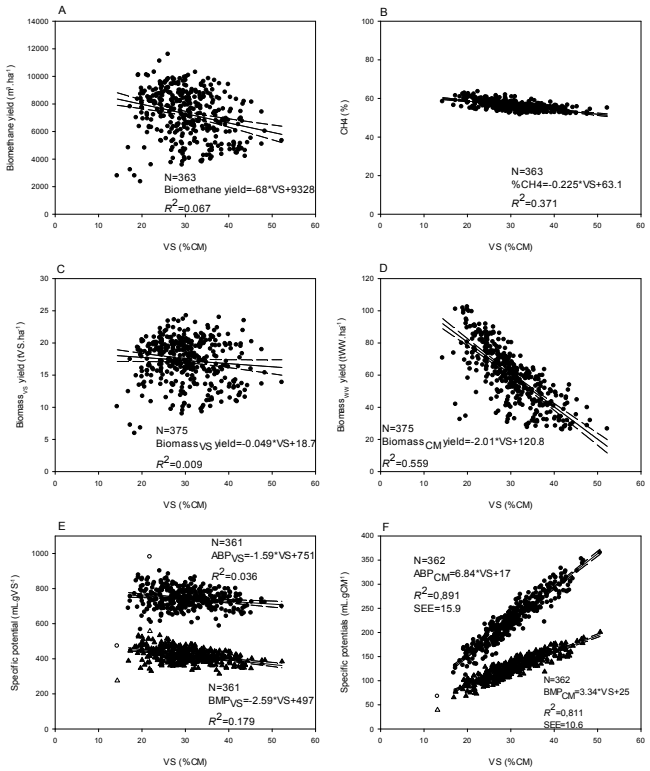

Figure 3.3. Linear regressions between the volatile solid (VS) content and various maize traits analysed for biomethanation: (A) biomethane yield per hectare, (B) methane content in the biogas, (C) $biomass_{VS}$ yield, (D) $biomass_{CM}$ yield, (E) anaerobic biogasification potential (ABP_{VS}) or biochemical methane potential (BMP_{VS}) on a volatile solids basis and (F) anaerobic biogasification potential (ABP_{CM}) or the biochemical methane potential (BMP_{CM}) on a crude matter (CM) basis. 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 3.3. Spearman's correlation coefficients between biomethanation traits of maize. Sampling distributions are not normally distributed. Significant correlations are marked with a star ($p < 0.05$).

Parameters	Biomethane yield ($m^3.ha^{-1}$)	Biogas yield ($m^3.ha^{-1}$)	$Biomass_{VS}$ yield ($tVS.ha^{-1}$)	$Biomass_{CM}$ yield ($tCM.ha^{-1}$)	Methane content ($\%CH_4$)	BMP_{VS} ($mL.gVS^{-1}$)	ABP_{VS} ($mL.gVS^{-1}$)	BMP_{CM} ($mL.gCM^{-1}$)	ABP_{CM} ($mL.gCM^{-1}$)	VS (%CM)
Biomethane yield ($m^3.ha^{-1}$)	1.00									
Biogas yield ($m^3.ha^{-1}$)	0.98*	1.00								
$Biomass_{VS}$ yield ($tVS.ha^{-1}$)	0.91*	0.93*	1.00							
$Biomass_{CM}$ yield ($tCM.ha^{-1}$)	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_{CM} ($mL.gCM^{-1}$))	-0.02	0.06	-0.03	-0.63*	-0.34*	0.02	0.22*	1.00		
ABP_{CM} ($mL.gCM^{-1}$)	-0.13*	-0.04	-0.10	-0.71*	-0.50*	-0.11*	0.14*	0.98*	1.00	
VS (%CM)	-0.29*	-0.19*	-0.11*	-0.77*	-0.60*	-0.38*	-0.16*	0.90*	0.95*	1.00

CM: crude matter, VS: volatile solids, ABP: anaerobic biogasification potential, BMP: biochemical methane potential.

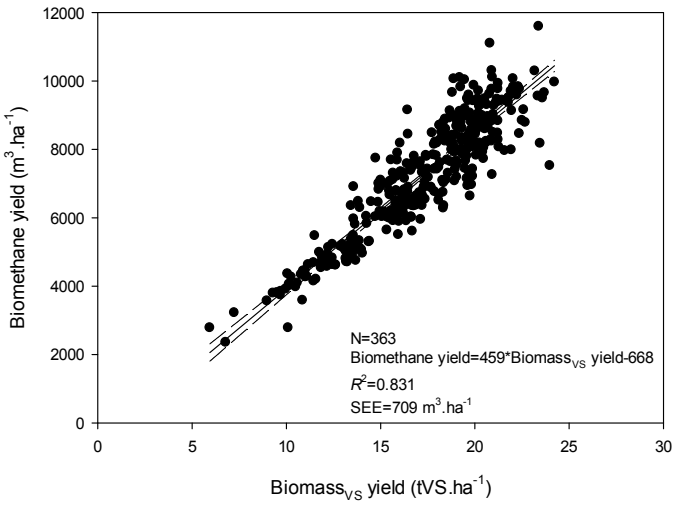

Figure 3.4. Linear regressions between biomass yield and biomethane yield for the maize samples analysed. Solid lines represent the linear regressions and dashed lines represent the 95% confidence bands. N: number of samples, R^2: coefficient of determination. SEE: standard error of estimate.

2.2. Factors influencing the biomass$_{VS}$ yield

One way of optimizing 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 3.2), indicating the existence of opportunities to optimize and maximize this parameter. Indeed, the biomass$_{VS}$ yield is the result of the product of the biomass$_{CM}$ 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$_{CM}$ yield and the VS (Table 3.2) respectively, offer large flexibility to alter both factors.

The correlation matrix (Table 3.3) shows a positive correlation (r = 0.67) between biomass$_{VS}$ yield and biomass$_{CM}$ yield, and a slight negative correlation (r = -0.11) between biomass$_{VS}$ yield and VS (also illustrated in Figure 3.3C). There is also a negative correlation (r = -0.77) between the biomass$_{CM}$ yield and VS (Table 3.3 and Figure 3.3D).

In most cases, the biomass$_{CM}$ yield decreased with late harvest dates (Figure 3.1). The only exception was in Corroy-le-Grand 2008 where the biomass$_{CM}$ yield remained stable over harvest dates. Late maturing maize varieties tended to produce more biomass$_{CM}$ than early maturing ones.

In other environments (Figure 3.2), late maturing maize varieties tended to yield more biomass$_{CM}$ with lower VS than early varieties. These trends are consistent because late maturing varieties need more time in the field to reach physiological maturity.

For all the groups (varieties x harvest dates) compared, the biomass$_{VS}$ yield did not differ significantly ($p < 0.05$) within an environment. The decrease of the biomass$_{CM}$ 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 & 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).

2.3. Factors influencing the BMP_{VS}

The BMP_{VS} of maize silages were on average higher in this study (Table 3.2, 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 3.2, mean: 743 $mL.gVS^{-1}$, RSD: 7.7%) and slightly decreases when VS increases (Figure 3.3E). The methane content also slightly decreases when VS increases (Figure 3.3B), with low variability around this trend (Table 3.2, RSD: 4.4%). The correlation matrix (Table 3.3), shows a high correlation coefficient between ABP_{VS} and BMP_{VS} ($r = 0.90$).

The trend of ABP_{VS} and %CH4 to decrease for increasing VS contents (Figure 3.3E and B) explain the trend of BMP_{VS} to decrease for increasing VS contents (Figure 3.3E).

Mature maizes with high VS content were characterized 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.

2.4. Characteristics of maize for biomethanation

Maize silages with lower VS tend to have slightly higher anaerobic digestibility (Figure 3.3E), higher methane content in the biogas (Figure 3.3B), and they

produced high biomass yield in the field (Figure 3.3D). Since the biomethane yield can be decomposed as the product of these factors (Equation 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 & Rath, 2012)

2.5. Prediction of ABP and BMP

Since moisture contained in maize does not contribute to biomethane production, relations between ABP_{CM} or BMP_{CM} and the VS content were investigated (Figure 3.3F). 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 3.3) between VS and both ABP_{CM} (r = 0.95) and BMP_{CM} (r = 0.90). The VS content explains most of the variability observed for ABP_{CM} (R^2 = 0.89) and BMP_{CM} (R^2 = 0.81) (Figure 3.3F). The ABP_{CM} and BMP_{CM} linearly increased with the VS content and could be modelled according to the equations in Figure 3.3F. The first-order linear regression allows then to predict ABP_{CM} and BMP_{CM} from the VS. Precision of these simple models (SEE of 15.9 and 10.6 $mL.gCM^{-1}$ for ABP_{CM} and BMP_{CM} respectively) appears to be good as compared to the accuracy of the reference method (SEL = 10 $mL.gCM^{-1}$ and 5 $mL.gCM^{-1}$ for ABP_{CM} and BMP_{CM} respectively, as determined in batch anaerobic digestion). Using these equations as predicting models can be a useful tool when considering the time needed, 42 to 56 days (Table 3.1) 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 optimize 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_{CM} and BMP_{CM} 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).

2.6. Precision of measurements in batch assays

The SEL values, which characterize the repeatability of the method, were 10 and 5 $mL.gCM^{-1}$ for ABP_{CM} and BMP_{CM} respectively, and 40 and 22 $mL.gVS^{-1}$ for ABP_{VS} and BMP_{VS} respectively (Table 3.2). The RSEL was around 4-5% for ABP_{CM}, ABP_{VS}, BMP_{CM} and BMP_{VS}. While the RSD values, which characterize the dispersion within the population, were 21.3% and 19.8% for ABP_{CM} and BMP_{CM}, 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_{CM} and BMP_{CM}.

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 this repeatability 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_{CM} and the BMP_{CM} 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.

3. 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 VS content, but suitable for silaging. To further improve 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_{CM} was linked to VS content and first-order linear regression allowed a quick prediction of both ABP_{CM} and BMP_{CM}.

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Annexe

Cropping design used for maize biomass production. Maize was grown and harvested in Corroy-le-Grand, Perwez and Roux-Miroir by the CIPF, and in Kehlen, Marnach, Nagem, Overpelt, Pletschterhof and Useldange by the ASTA. SD: sowing date, HD: harvest date, FR: field replicates, N: number of samples. The FAO maturity class is given between brackets following the variety name.

Environment (year-field)	SD	HD	Variety (FAO index)	FR	N
Corroy-le-Grand 2007	03/05/2007	11/10/2007 17/10/2007 26/10/2007	Asteri (230), DKC 3745 (220) Graphic (220), LG 3277 (250), Maïbi (240)	2	30
Corroy-le-Grand 2008	13/05/2008	24/10/2008 31/10/2008 06/11/2008	Atletico (280), Lucatoni (340), Piazza (240), Seiddi (300)	4	48
Kehlen-2008[1]	05/05/2008	26/09/2008 03/10/2010	Atletico (280), Lucatoni (340), Piazza (240), Seiddi (300)	1	7
Marnach 2008	08/05/2008	15/10/2008	Atletico (280), Piazza (240)	1	2
Nagem 2008	13/05/2008	13/10/2008	Atletico (280), Lucatoni (340), Peru (900), Piazza (240), Seiddi (300)	1	5
Overpelt 2008	25/04/2008	24/09/2008	0808HYB (220), 0881HYB (250), Aabsolut (240), Arting (250), Asteri (230), Atletico (280), ES Charles (245), ES Surplus (250), Franki (250), Ginko (320), Piazza (240), Seiddi (300), Sumaris (240)	3	39
Perwez 2008	26/04/2008	07/10/2008	0808HYB (220), 0881HYB (250), Aabsolut (240), Arting (250), Asteri (230), Atletico (280), ES Charles (245), ES Surplus (250), Franki (250), Ginko (320), Lucatoni (340), Piazza (240), Seiddi (300), Sumaris (240)	3	42
Pletschterhof 2008	07/05/2008	03/10/2008	Atletico (280), Lucatoni (340), Piazza (240), Seiddi (300)	1	4
Useldange 2008	06/05/2008	24/09/2008	Atletico (280), Lucatoni (340), Piazza (240), Seiddi (300)	1	4
Corroy-le-Grand 2009[2]	22/04/2009	16/09/2009 22/09/2009 30/09/2009 05/10/2009	Atletico (280), Lucatoni (340), Piazza (240), Seiddi (300)	4	63
Kehlen 2009	05/05/2009	30/09/2009 16/10/2009 27/10/2009	Atletico (280), Lucatoni (340), Piazza (240), Seiddi (300)	4	48
Roux-Miroir 2009	01/05/2009	23/09/2009	0945HYB (240) , Aapple (250), Atletico (280), DKC3390 (235), Jessy (230), Lucatoni (340), Marleen (240), Seiddi (300), Subito (260)	4	36
Useldange 2009[3]	30/04/2009	29/09/2009 16/10/2009 27/10/2009	Atletico (280), Lucatoni (340), Piazza (240), Seiddi (300), Peru (900)	4	51

[1] Variety Piazza harvested on 26/09/2008 misses.
[2] First replicate of variety Piazza harvested on 30/09/2009 misses.
[3] Variety Peru had no replicate.

Highlights of Chapter 3

- The cropping environment is the main cause of variability of biomethane yield of maize silages.
- The biomass yield is the main factor driving the biomethane yield of maize silages.
- The VS content can predict the ABP_{CM} and BMP_{CM} of maize silages.
- Late maturing maize varieties harvested at an early stage are probably best to reach high biomethane yield because they yield high biomass with high anaerobic digestibility.

Chapter 4

Biochemical composition of maize silages and its prediction with models using near infrared spectroscopy

Wet maize silages have been produced under various agro-climatic conditions and analyzed for their BMP as wet substrates (Chapter 3). During the anaerobic digestion process, biomethane is produced through the degradation and conversion of the organic molecules of the substrate. The VS content was shown to influence the BMP_{CM} of wet maize silages. On the other hand, NIRS has been reported to be a suitable analytical technology to characterize the organic matter of the biomass. **What is the biochemical composition of maize silages used in anaerobic digestion and how are models based on NIRS able to predict this biochemical composition?**

In the present chapter (Chapter 4), the biochemical composition of the maize silages was assessed. Models that predict the biochemical composition with NIRS were investigated as a preliminary step to the prediction of the BMP.

Abstract

The biochemical composition of maize silages was measured to assess the variability of the main biochemical components and the correlations between components of the crude matter (CM). Wet maize silages have a low content in ash (1.35 ± 0.22 %CM), which allows their characterization as a bi-component biomass constituted of volatile solids (VS) and moisture. Starch and cellulose were respectively correlated and anti-correlated with the volatile solid content. The biochemical composition was predicted using near infrared spectra measured either on wet silages (wet NIRS) or on dry and ground silages (dry NIRS). Wet NIRS proved to be fast and useful to accurately predict the moisture and VS contents (standard error of prediction: 1.1 %CM for both parameters). On the other hand, dry NIRS provided better prediction of the biochemical composition, especially in terms of cellulose, hemicellulose, crude proteins and fat (root mean square error of cross-validation of 1.0, 1.1, 0.49 and 0.22 %TS respectively). Important spectral ranges for the prediction of the measured compounds were between 5400 and 4300 cm^{-1}. Considering the ability of NIRS to predict the biochemical composition of maize silages, it is expected to have a good ability to estimate the biochemical methane potential of such a biomass.

Keywords: fibres, cellulose, hemicellulose, lignin, starch, proteins, fat, near infrared spectroscopy.

1. Introduction

Anaerobic digestion consists in a bioprocess where various microorganisms degrade and convert organic materials into a biogas that mainly contains methane and carbon dioxide (Mara & Horan, 2003). Feedstock of anaerobic digesters is very diverse and mainly consists in animal effluent, industrial or agricultural wastes and co-products, marine biomass and energy crops. The biochemical composition of the feedstock is variable and affects the methane content in the produced biogas (Baserga, 1998). Inappropriate feedstock may lead to bioprocess inhibition and failure (Chen et al., 2008). For instance, digestion of highly fermentescible matter like glucose produces quickly a high amount of intermediary compounds, especially volatile fatty acids, which inhibit the process when produced in large amounts (Marchaim & Krause, 1993). Digestion of proteins, urea and nucleic acids produce ammonia that may also inhibit the anaerobic digestion process if generated in inappropriate quantities (Rajagopal et al., 2013). The knowledge of the biochemical composition of a feedstock is thus necessary to properly manage anaerobic digesters and biogas production.

In agricultural biogas plants, energy crops are used as a substrate complementary to animal effluents. Energy crops are lignocellulosic biomass mainly made of structural carbohydrates (mostly cellulose and hemicellulose) with some lignin. Non-structural carbohydrates are principally starch and soluble sugars.

The chemical methods used to characterize the biochemical composition of biomass are usually time-consuming, require various analytical devices and produce chemical wastes. Models based on near infrared spectroscopy (NIRS) have been shown for decades to be fast and accurate methods to predict the biochemical composition of various biomass (Burns & Ciurczak, 2007) and particularly maize (Dardenne et al., 1993). However, most NIR measurements are made on plant material that is preprocessed (freeze drying, heat drying, milling, etc.). Indeed, the increase of particle comminution (Lovett et al., 2005) or the oven drying (Sørensen, 2004) were shown to improve calibration statistics of prediction models based on NIRS. Such preprocessings improve accuracy of final results but require time and delay the achievement of results, while increasing the analysis cost. Avoiding the

preprocessing step allows faster prediction and on-line application (Holm-Nielsen & Esbensen, 2011; Jacobi et al., 2011).

The prediction of biochemical composition (fibres, proteins, fat) or characteristics (enzymatic digestibility) of maize silages from NIR spectra of both raw and preprocessed material has been broadly investigated. The comparison of prediction models between NIR spectra measured on raw wet matter or on preprocessed matter was less considered (Sørensen, 2004; Park et al., 2005).

The goal of this paper is first to assess the variability and correlations of the biochemical compounds of maize silages used for biomethanation. NIR spectra measured either on wet matter or on dry and ground matter will be also investigated to create prediction models of the biochemical composition of maize silages. Precision of the biochemical reference method and the importance of spectral range in the prediction model will be assessed.

This work constitutes a preliminary step towards the evaluation of NIRS for the prediction of biochemical methane potential (BMP) of maize silages, which is reported to be influenced by the biochemical composition (Grieder et al., 2011; Rath et al., 2013).

2. Material and methods

2.1. Biomass production

Maize samples were cropped in Useldange in 2010 (varieties Atletico, Lucatoni, Piazza, Seiddi) and in Nagem in 2011 (varieties Atletico, LG 30238, Lucatoni, and Seiddi) by the *Administration des Services Techniques de l'Agriculture* (ASTA) in Luxembourg. In the environment (field x year) of Useldange 2010, maize was sown at a single date and harvested at three different dates with an interval of two weeks. In Nagem 2011, maize was sown at three different dates with an interval of one week, but harvested at a single date. Two field replicates were harvested at each harvest date for each variety. One maize sample (variety Subito) cropped in Useldange in 2012 was also harvested. This crop design was used with the aim to create variability among the maize samples in terms of maturity and biochemical composition.

In addition to these 49 maize samples, 379 maize silages produced in 2007, 2008 and 2009 in Luxembourg and in Belgium, and described previously (Mayer et al., 2014), were included in the present study, leading to a total number of 428 maize samples.

The maize samples were ensiled under vacuum in sealed plastic bags and stored at room temperature until laboratory analysis (Mayer et al., 2014).

2.2. Biochemical measurements

The moisture, the volatile solids (VS) and the ash contents were measured for the wet maize silages. Moisture was defined as the weight lost by the wet matter after a drying step in an oven at 105°C for 24h. The remaining dry matter, called total solids (TS), was subsequently burned in a furnace at 550°C for 6h. The loss of mass between the TS and the ash was defined as the volatile solids (VS). Moisture, VS and ash were measured on sub-samples of approximately 30-40 g of wet maize silages.

A subsample of wet maize silages was dried in an oven at 70°C for 48h and ground through a 1-mm mesh in a centrifugal mill (Cyclotec, Foss) to obtain a dry maize silage powder. This powder was used thereafter to characterize the biochemical composition of maize silages in fibres (hemicellulose, cellulose and lignin), fat, crude proteins (CP) and elemental C and for NIRS measurements.

Fibres analysis was carried out using methods adapted from Van Soest (Van Soest et al., 1991) and modified for starch removal (Mertens, 2002). Briefly, 0.5 g of powder was sealed in a filter bag and successively incubated in different solutions to measure the neutral detergent fibres (NDF) (Ankom, 2006a) and the acid detergent fibres (ADF) (Ankom, 2006b) with an Ankom automatic analyser (Ankom 2000, Ankom Technology). The acid detergent lignin (ADL) (Ankom, 2005) was measured after digestion in a beaker. After each digestion, moisture was removed through soaking of the bags in acetone followed by a drying step in an oven at 105°C. After the drying step, bags were cooled to room temperature in a desiccator. NDF, ADF and ADL were quantified gravimetrically by weighting the digested residues in the filter bag. Hemicellulose was calculated as the difference between NDF and ADF, and cellulose as the difference between ADF and ADL. The ADL values were not corrected for the ash content.

The fat content of dry maize silage powders was determined with adapted standard method (AOAC International, 2006). A subsample of 5 g of dry maize silage powder, contained in a cellulose thimble, was washed for 30 cycles into n-hexane (Biosolve) using a Soxhlet extractor (Buchi). The fat was then separated from the solvent with a rotary evaporator and the fat residue was quantified gravimetrically.

Elemental C and N were measured from 0.5 g of dry maize silage powder with a TruSpec elemental analyser (LECO) and expressed as a percentage of the TS. The crude proteins (CP) were calculated by multiplying the elemental N value by 6.25 (Jones, 1931).

The starch content was predicted by the *Centre wallon de recherches agronomiques* (CRA-W, Gembloux, Belgium) with a validated model using near infrared spectra of the dry maize silage powders.

All biochemical analyses were carried out in triplicates.

2.3. NIRS measurements

Near infrared (NIR) spectra were acquired on both wet maize silages (wet NIRS) and dry maize silage powders (dry NIRS). NIR spectra were measured in an air-conditioned laboratory (23°C) with a Bruker MPA spectrometer (Bruker Optik GmbH) operated with OPUS 6.5. The spectrometer was equipped with an integrating sphere for reflectance measurements. Before each measurement session, the spectrometer was validated according to the procedure installed in the software (PQ test). A background measurement was performed before the first measurement and each hour of the measurement sessions.

For the NIR spectrum acquisition, a hard coated aluminium sample cup of 97 mm diameter with a water free quartz window (IN 312-S) was filled with the sample, before being placed on a rotating sample cup holder (IN 312/C). The window of the spectrometer (diameter of 2.5 cm approximately) was not aligned with the centre of the circular sample cup in order to scan a representative heterogeneous surface (a circular crown of around 35 cm^2) during the rotation of the cup. Absorbance spectra were collected in the 3594-9989 cm^{-1} range with a resolution of 16 cm^{-1}. The measured NIR spectrum of a sample was the result of the average of 32 spectra automatically collected during the rotation of the sample cup. For each sample, three NIR acquisitions were realized after emptying, cleaning and repacking the sample

cup with new material, to obtain three NIR spectra corresponding to one single silage sample.

2.4. Data treatment and chemometrics

For cultivated plants, the calibration set should be ideally split from the validation set according to the harvest year (Davies, 2004; Dardenne, 2010). Maize silages harvested in 2007, 2008 and 2009 were used in the calibration set to create and assess the prediction models through cross-validation. Maize samples cropped in 2010, 2011 and 2012 constituted the validation set to test the models without any influence of the calibration procedure.

Descriptive statistics were calculated 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 biochemical parameters were measured with the Spearman's correlation coefficient.

Before any model calculation, the feasibility of a NIR model development was assessed according to Dardenne (2010). The standard error of laboratory (SEL) of a measured parameter was calculated according to:

$$SEL = \sqrt{\frac{\sum s}{N}}$$

with *s*, the variance of the replicate analysis for one sample and *N* the number of samples in the dataset.

The maximum coefficient of determination of any calibration (R^2_{max}) was calculated using:

$$R^2_{max} = \frac{SD^2 - SEL^2}{SD^2}$$

with *SD*, the standard deviation of the dataset for the reference value.

Multivariate data analysis was carried out with Matlab, version R2012a (MathWorks) and PLS_Toolbox, version 7.3.1 (Eigenvector Research, Inc.).

Variability of biochemical composition of samples and correlations between samples and biochemical parameters were analyzed with a principal component analysis (PCA). Data were autoscaled before calculating the PCA.

For the prediction model calculation, the triplicate NIR spectra of a sample were averaged to obtain one single spectrum per sample. Triplicate and average spectra

were plotted to check outliers before being used in the model calculation as predictors. In each model calculation and for each reference parameter, the calibration set and the validation set were described with the total number of samples, the number of outliers, the minimum, mean and maximum values, and the standard deviation. The regression method used was the partial least squares (PLS) for all models.

For the spectral data, preprocessing methods included mean centering (MC), autoscaling (AS), standard normal variate (SNV) or detrend (Det). Derivatives were also assessed and abbreviated as xD(y;z), with x the derivative order, y the polynomial order and z the filter width. The excluded spectral data were used in the derivative calculations, but not in the subsequent model.

A cross-validation was used during the calibration procedure to find the optimal number of latent variables (LV) for multivariate models. The cross-validation consisted in the random split of the calibration set in 10 segments, with same number of samples in each segment. The calibration model was then calculated with all the samples from 9 segments. Using this calibration model, the value of the parameter was predicted for each sample of the 10^{th} segment. This procedure was repeated 10 times to calculate the statistics of the model.

The root mean square error (RMSE), the coefficient of determination (R^2) and the ratio of standard error of prediction to sample standard deviation (RPD) were calculated for the calibration (RMSEC, R^2C, RPDC), cross-validation (RMSECV, R^2CV, RPDCV), and validation (RMSEP, R^2P, RPDP).

The RMSE was calculated according to:

$$RMSE = \sqrt{\frac{\sum(y_m - y_p)^2}{N}}$$

with y_m the measured values, y_p the predicted values and N the number of samples in the dataset.

The R^2 was calculated according to:

$$R^2 = \left(\frac{\sum(\widehat{y_m} - \overline{y_m})^2}{\sum(y_m - \overline{y_m})^2}\right)$$

with y_m the measured values, $\widehat{y_m}$ the estimated y_m values given by the regression line and $\overline{y_m}$ the mean y_m value.

The RPD, which is a more discriminant value than the R^2 value, was calculated according to:

$$RPD = \frac{1}{\sqrt{1 - R^2}}$$

For the validation sets, the bias, corresponding to the systematic averaged deviation between the dataset of the true and the predicted values (Conzen, 2006), was calculated according to:

$$Bias = \frac{1}{N}\sum(y_m - y_p)$$

with y_m the measured value and y_p the predicted value.

The standard error of prediction (SEP), which is the RMSEP corrected for bias, was calculated according to

$$SEP = \sqrt{\frac{1}{N-1}\sum(y_m - y_p - bias)^2}.$$

The intercept and the slope were calculated for the linear regression between the predicted values (x-axis) and the measured values (y-axis) for the validation or the cross-validation data. The best model was chosen and presented according to the lowest RMSECV, the lowest number of latent variables (LV) and highest R^2CV and RPDCV.

The variable importance in projection (VIP) scores give a summary of the importance of a predictor for both predictors and predicted parameter. A variable with a VIP score close to or greater than 1 can be considered as important in a given model (Wold et al., 2001). The VIP score for the *j*-th variable was calculated according to:

$$VIP_j = \sqrt{p\frac{\sum_{k=1}^{h}\left(b_k^2 t_k^t t_k \left(\frac{w_{jk}}{\|w_k\|}\right)^2\right)}{\sum_{k=1}^{h} b_k^2 t_k^t t_k}}$$

with p the number of predictors, h the number of LV, k the k-th LV, b the regression coefficient of the score matrix, t the column vector of the score matrix, w the column vector of the weight matrix (Chong & Jun, 2005).

For the calibration set and the validation set, the SEL of prediction of each parameter was calculated by applying the model to NIR spectra replicates in order to obtain the predicted values.

3. Results and discussion

3.1. Maize composition

The biochemical composition of maize silages was completely described for the calibration set, whereas only the moisture, VS and ash contents were measured in the validation set, as presented in Table 4.1.

Table 4.1. Biochemical composition of maize silages for the calibration set and the validation set.

Factors Unit	Moisture (%CM)	VS (%CM)	Ash (%CM)	Hemicellulose (%TS)	Cellulose (%TS)	ADL (%TS)	CP (%TS)	C (%TS)	Fat (%TS)	Starch (%TS)
Calibration set										
N	375	375	375	357	357	338	355	355	264	354
Min	46.2	14.3	0.72	12.4	11.6	0.49	5.1	44.3	0.89	4.1
Max	84.8	52.3	1.96	30.5	36.1	3.72	10.3	47.0	6.00	43.7
Range	38.6	38.0	1.24	18.1	24.5	3.24	5.2	2.7	5.11	39.6
Mean	68.3	30.3	1.35	16.7	19.6	1.47	7.1	45.6	2.70	31.0
SD	6.7	6.6	0.22	2.2	3.2	0.50	1.1	0.53	0.57	6.8
RSD (%)	9.8	21.7	16.0	13.0	16.3	33.9	14.8	1.2	21.1	21.9
SEL	0.88	0.90	0.07	0.93	0.72	0.24	0.07	0.16	0.20	0.8
RSEL (%)	1.3	3.0	5.4	5.5	3.7	16.1	0.95	0.36	7.4	2.5
SEL/SD	13.2	13.6	33.4	42.6	22.7	47.7	6.4	31.1	34.8	11.6
R^2_{max}	0.98	0.98	0.89	0.82	0.95	0.77	0.99	0.90	0.88	0.99
Validation set										
N	49	49	49	-	-	-	-	-	-	
Min	61.4	18.8	0.74	-	-	-	-	-	-	
Max	80.4	37.3	1.35	-	-	-	-	-	-	
Range	19.0	18.5	0.61	-	-	-	-	-	-	
Mean	70.8	28.2	0.98	-	-	-	-	-	-	
SD	4.7	4.6	0.14	-	-	-	-	-	-	
RSD (%)	6.7	16.5	14	-	-	-	-	-	-	
SEL	0.59	0.59	0.03	-	-	-	-	-	-	
RSEL (%)	0.8	2.1	3.2	-	-	-	-	-	-	
SEL/SD	12.4	12.8	23.3	-	-	-	-	-	-	

Starch was predicted with near infrared spectroscopy by the CRA-W (Gembloux, Belgium). All other parameters were measured with wet chemistry. ADL: acid detergent lignin, C: elemental C, CM: crude matter, CP: crude proteins, N: number of samples, R^2_{max}: maximum coefficient of determination, RSD: relative standard deviation, RSEL: relative standard error of laboratory, SD: standard deviation, SEL: standard error of laboratory, TS: total solids, VS: volatile solids.

The three main fractions (moisture, VS and ash) of maize silages were measured on 375 samples (Table 4.1) in the calibration set. The main composition parts of maize were the moisture (68.3 ± 6.67 %CM) and the VS (30.3 ± 6.57 %CM) representing

together at least 98 %CM of the wet maize silages. Ash represented a maximum of 1.96 %CM with an average of 1.35 ± 0.22 %CM. Considering the very low amount of ash, wet maize silages analyzed in the present study can be described as bi-component biomass made of VS and moisture. Knowing the moisture allows thus to approximate the VS content.

The repeatability of the ash measurement (RSEL: 5.4 %CM) was lower than the repeatability of the moisture (RSEL: 1.3 %CM) and VS (RSEL: 3.0 %CM) measurements (Table 4.1). This high RSEL for ash measurements can be attributed to the gravimetric reference method. While the method was suitable to accurately weight the wet (around 30-40 g) and dried silage subsamples (around 10 g), the method was less accurate when measuring the amount of ash that remains after combustion of the dried silage (less than 1 g). Another gravimetric method, allowing the measurement of ash content from a higher quantity of wet or dry material, could improve the analysis results but would need large furnace and more biomass. The maximum coefficient of determination was lower for ash (R^2_{max} of 0.89, Table 4.1) than for moisture and VS (R^2_{max} of 0.98 for both) because the repeatability of the ash measurement was low within a tight range of observations.

Starch, measured with NIRS, was the main constituent of the dry matter of maize silages (Table 4.1, 31.0 ± 6.8 %TS). Starch is present in maize grains and increases along with maturity (Schittenhelm, 2008). The variability of starch (RSD of 21.9%) shows that a large range of maturity was available in the dataset.

The hemicellulose and cellulose analyses were retained for 357 different silage samples. Fewer results (N = 338) were validated for the ADL measurement because of the variability within replicates and the lack of biomass to reanalyse some of the samples.

Hemicellulose and cellulose were almost equally present in maize silages with 16.7 ± 2.2 %TS and 19.6 ± 3.2 %TS respectively (Table 4.1), whereas ADL constituted the lowest fraction of fibres (1.5 ± 0.5 %TS). The RSEL was high for ADL (16.1%), as compared to hemicellulose (5.5%) and cellulose (3.7%). The repeatability of strong acidic digestions in beakers to measure ADL was lower than the one of NDF and ADF methods, which used an automatic device to solubilize digestible compounds. Such high SEL within the measured range of ADL values provided to ADL prediction the lowest maximum coefficient of determination of all measured

parameters, with a R^2_{max} of 0.77. Some improvement, such as a continuous mixing of the ADL digestion solution, must be considered to decrease the SEL of ADL measurement and hypothetically improve prediction models of this biochemical fraction.

The elemental C and N (converted into CP) were measured on 355 maize silage samples (Table 4.1). Little variability was observed for elemental C (45.6 ± 0.53 %TS) in maize silages, as shown by its RSD (1.2%). The elemental C does not reflect a particular fraction of the biochemical composition, but is used to determine the C:N ratio of the feedstock of anaerobic digesters, that should be in the range of 16:1-25:1 as an indication (Deublein & Steinhauser, 2008). For the varieties and maturity range considered in the present study, the analysis of elemental C is not of major interest for maize silage characterization because of its low variability.

The CP was less abundant (7.1 ± 1.1 %TS) than starch, hemicellulose or cellulose in maize silages. A high R^2_{max} was determined because of the accurate CP measurements (RSEL: 0.95%) and high variability (RSD: 14.8%) in maize silages. A high accuracy is expected for models predicting the CP content of maize silages included in the presented set.

The fat was measured on a slightly lower number of samples (N = 264) because the method was highly time-consuming for a single sample and required significant amount of a hazardous solvent. Fat was not highly present in maize silages (2.7 ± 0.6 %TS), as compared to starch, hemicellulose, cellulose or CP, but showed a high variability (RSD: 21.1%) within maize silage samples. However, the RSEL for fat was high (7.4%). The analytical method should be improved to reduce the variability between replicates.

All biochemical compounds of maize silages were not analyzed in the present study. On average, the biochemical analysis characterizes 78.6% of the total solids of maize silages. A significant part of the TS remained unknown, but a similar biochemical composition, representing 73.9 %TS on average, was already reported when cumulating starch, NDF, CP and fat (Grieder et al., 2011).

A part of the unknown fraction of TS can be explained by unmeasured compounds such as some soluble sugars, organic acids and alcohols (Leduc, Fournier, Payant, & Blais, 1997). Remaining non-identified fraction (around 20 %TS) is made of other

compounds that were not extracted in the different methods used in the present study.

Bias between the true and the measured values can also exist due to the analysis method. For instance, the CP was calculated by multiplying the N content, measured through elemental analysis, by the coefficient 6.25. Ingredient-specific conversion factor could be considered (Sriperm et al., 2011) for CP measurement. The influence of high amount of starch on NDF estimation through Van Soest digestion has already been reported (Giger-Reverdin, 1995). The lignin content can be underestimated with the ADL measurement resulting from the Van Soest successive digestions (Goff et al., 2012). Such misestimations can explain a part of the undetermined fraction of maize silages described in the present study.

3.2. Correlations between biochemical fractions

Spearman's correlation coefficients were calculated between the measured biochemical parameters (Table 4.2). A PCA was performed with the 10 biochemical parameters measured on the ensiled maize samples to further explain the correlations between biochemical parameters and the samples. Samples for which the composition analysis was incomplete were not included in the PCA in order to have no missing data. The PCA was thus calculated with 235 samples and the main results are presented in Figure 4.1.

The data used in this PCA were measured on the wet maize silages (moisture, VS and ash contents) and on the dried and ground maize silages (cellulose, hemicellulose, lignin, C, CP, fat and starch contents). Despite the fact that two PCA with each type of measurement would make sense from a biological point of view, such an approach showed similar results about the correlations between parameters measured on one maize form (data not shown), but it did not shown informations between the moisture, VS and ash contents and the other organic fractions. The results of a unique PCA are thus presented.

A total of 6 PC was required to account at least for 94% of the variance (Figure 4.1A). The first PC (PC1) accounted for 47% of the total variability and mainly discriminates VS from moisture (Figure 4.1D). These two parameters were totally anti-correlated (Table 4.2, r = -1.00). PC1 captured also most of the variance of the cellulose and starch, which were anticorrelated (r = -0.71) and correlated (r =

0.66) to VS, respectively. Variances of hemicellulose and ADL were also mainly captured by PC1. Hemicellulose, cellulose and ADL, representing the fibre fraction, were highly correlated along the PC1 and according to Spearman's coefficients (Table 4.2), as already observed by other authors (Cozzolino et al., 2006; Liu & Han, 2006). Considering these correlations, the VS content of maize silages gathers information about starch and fibres that were the most abundant measured fractions in the biochemical composition of this energy crop. For wet maize silages, the VS content can thus described the biochemical composition of the organic matter.

The PC3 discriminated two groups, characterized by the country where samples were harvested (Figure 4.1C). This PC was related to ash, CP and elemental C, according to the variance captured per PC (Figure 4.1B) and the loading plot (Figure 4.1D). On average, the ash and CP contents were higher for samples harvested in Belgium than for those from Luxembourg, while the VS content was higher for samples harvested in Luxembourg (Figure 4.1E). The maturity of maize can be characterized by the VS content and has an effect on the ash and CP contents (Schittenhelm, 2008). The maturity at the harvest of maize silages could be different in Luxembourg and in Belgium and could explain the different contents in ash and in CP. The pedoclimatic conditions or the difference in the abundance and availability of nitrogen for maize in Luxembourg and Belgium could be also responsible for the discrimination observed along PC3, since N input has an effect on N yield (Herrmann, 2012).

Table 4.2. Spearman's correlation coefficients between biochemical parameters of maize silages.

Parameters	Moisture (%CM)	VS (%CM)	Ash (%CM)	Hemicellulose (%TS)	Cellulose (%TS)	ADL (%TS)	CP (%TS)	C (%TS)	Fat (%TS)	Starch (%TS)
Moisture (%CM)	1.00									
VS (%CM)	-1.00*	1.00								
Ash (%CM)	-0.46*	0.43	1.00							
Hemicellulose (%TS)	0.32*	-0.32*	-0.06	1.00						
Cellulose (%TS)	0.70*	-0.71*	-0.05	0.64*	1.00					
ADL (%TS)	0.52*	-0.53*	0.00	0.58*	0.70*	1.00				
CP (%TS)	0.47*	-0.47*	-0.03	-0.02	0.27*	0.25*	1.00			
C (%TS)	0.21*	-0.20*	-0.41*	-0.04	0.03	0.03	0.27*	1.00		
Fat (%TS)	-0.14*	0.15*	-0.13*	-0.56*	-0.44*	-0.38*	0.26*	0.20*	1.00	
Starch (%TS)	-0.65*	0.66*	0.16*	-0.62*	-0.77*	-0.55*	-0.23*	-0.02	0.49*	1.00

Sampling distributions are not normally distributed. Significant correlations are marked with a star ($p < 0.05$). ADL: acid detergent lignin, C: elemental C, CM: crude matter, CP: crude proteins, TS: total solids, VS: volatile solids.

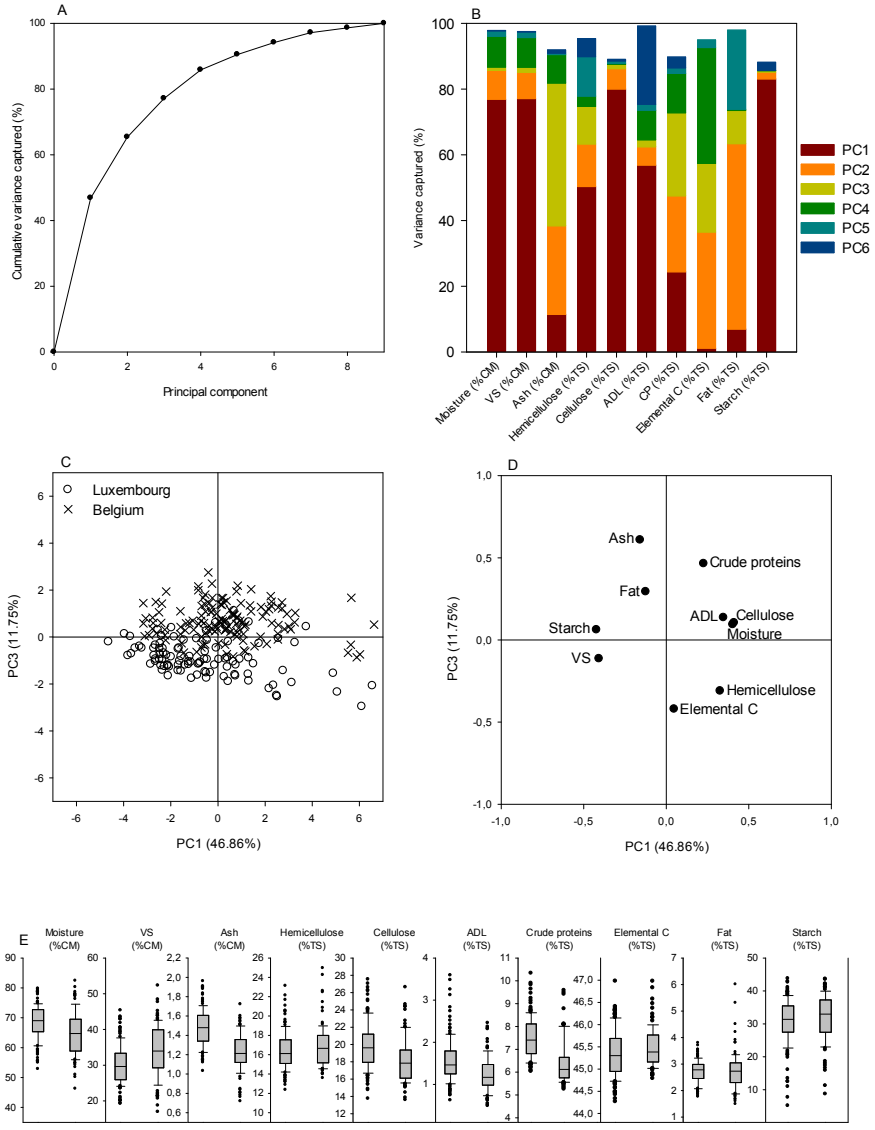

Figure 4.1. Cumulated variance captured (A), variance captured per principal component for each variable (B), scores plot (C) and loadings plot (D) of the principal component analysis (PCA). Boxplots of biochemical parameters are split according to the harvest country (E: Belgium, left; Luxembourg, right).

3.3. Prediction models from NIRS

Mean of the near infrared spectra of the wet maize silages (wet NIRS) and of the dry maize silage powders (dry NIRS) are shown in Figure 4.2. A sub-range (9002-3903 cm^{-1}) of the total measured spectral data was included in the modelling procedure to improve prediction results.

The absorbance of wet NIRS was higher than dry NIRS and characteristed by two peaks at 6873 and 5176 cm^{-1}. The water present in wet maize silages (around 70 %CM, Table 4.1) is responsible of such a profile for wet NIRS (Conzen, 2006).

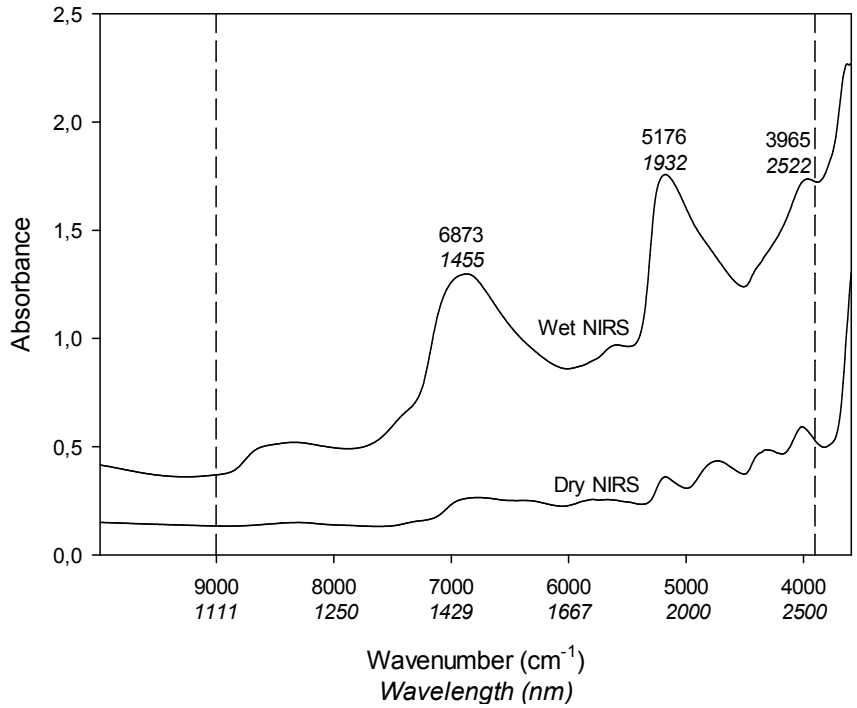

Figure 4.2. Mean of near infrared spectra of wet maize silages (wet NIRS) and of dry maize silage powders (dry NIRS). Dashed vertical lines represent the range (9002-3903 cm^{-1}) included in the modelling procedure.

Prediction of moisture, VS and ash from wet NIRS

The reference values of the validation set were in the same ranges as those observed for the calibration set (Table 4.1). The validation set was fully suitable to assess the models predicting the moisture, VS and ash contents (Conzen, 2006).

The results of calibration and validation for the models that predict moisture, VS and ash contents with wet NIRS or dry NIRS are presented in Table 4.3. The measured and predicted values for the validation set are shown in Figure 4.3. The VIP scores corresponding to each model are presented in Figure 4.4.

The models predicting moisture and VS contents using wet NIRS (Table 4.3) were accurate as indicated by RPD higher than 4.4, a SEP of 1.1 and R^2P of 0.95 for both parameters, with slopes of 1.03 and 1.04 for the graphs presenting measured vs. predicted value of moisture and VS respectively (Figure 4.3). Other authors (Reeves et al., 1989; Sørensen, 2004; Liu & Han, 2006) reported similar good results for the prediction of dry matter (or oppositely moisture) in maize. For moisture and VS contents, the repeatability (SEL of 0.88 %CM and 0.90 %CM, Table 4.1) and the error of prediction (SEP of 1.1 %CM) were close. The measurement of the moisture and VS contents in wet maize silages from wet NIR spectra is thus fully suitable for a routine measurement method.

VIP scores of models for moisture and VS from wet NIRS (Figure 4.4) have similar profiles and peaks. Moreover, the most important variable for these models are in the spectral range included between 5200 cm^{-1} and 5400 cm^{-1}. Absorbance signals of water are in this range (Conzen, 2006). Since wet maize silages can be described as a bi-component product made of VS and moisture, both models use the information about water measured by the NIR spectrometer to calculate the moisture content on one hand and the VS content, as the complement of moisture, on the other hand.

The RPDP of models that predict the ash content was comprised between 1.1 and 1.5 (Table 4.3). The prediction of the ash content was poor with both spectral predictors. The lower R^2_{max} of 0.89 for ash content, as compared to the one for moisture or VS (Table 4.1), indicated that the ash prediction would be less accurate than the prediction of moisture or VS contents. Cozzolino (2006) also did not succeed to predict accurately ash in wet maize silages, whereas others authors succeeded in dry maize silages (Liu & Han, 2006). Considering the method using a furnace and the gravimetric measurement to quantify the ash content in maize

silages, a higher weight of ashes remains after incinerating dry biomass than after incinerating the same weight of wet biomass. The reference method can have an effect on the accuracy of the prediction model.

Biases higher than 0.30 %CM, corresponding to 31% of the average ash content of the validation set, were found for both models predicting ash (Table 4.3). Biases were also shown in Figure 4.3. It appears that the validation set included only samples from Luxembourg. Samples harvested in Luxembourg have lower contents in ash and crude proteins than those harvested in Belgium (Figure 4.1E). Such specificity of the validation set could explain the high biases. Other models were calibrated with samples harvested only in Luxembourg (data not shown). However with such models, the biases of models using wet NIRS or dry NIRS were not reduced.

The R^2_{max} was high for moisture and VS contents and lower for the ash content (Table 4.1). The R^2C was found to be high after the modelling procedure for moisture and VS contents whereas the prediction of ash from NIRS was not accurate (Table 4.3). The determination of the R^2_{max} value is then valuable to state if a prediction model will be feasible or not and thus avoid time-consuming modelling procedure.

Since humidity was removed from the dry maize silage powders used to measure dry NIRS, the prediction of the moisture or VS content with dry NIRS makes no sense from a practical point of view and will not be used. However, the RPDP of dry NIRS models that predict the moisture or VS content was 1.7 and the slope of the linear regression was close or equal to 1.00 (Table 4.3). Some information about the moisture content of the wet biomass was present in dry NIRS models. A peak at 4443 cm-1 is observed on the VIP scores of dry NIRS models that predict the moisture or VS content (Figure 4.4). Similar high VIP score is observed for dry NIRS model that predicts the cellulose content (Figure 4.7). Correlations between the VS or moisture content and an organic fraction such as cellulose can explain the ability of dry NIRS models to predict information about the wet maize.

Table 4.3. Prediction models of moisture, volatile solids (VS) and ash contents in wet maize silages with near infrared spectroscopy (NIRS).

Calibration and cross-validation						
Substrate	Wet maize silage					
Predictor	Wet NIRS			Dry NIRS		
Parameter	Moisture	VS	Ash	Moisture	VS	Ash
Unit	(%CM)	(%CM)	(%CM)	(%CM)	(%CM)	(%CM)
N	371	371	371	357	357	357
Outliers	0	0	0	0	0	0
Minimum	46.2	14.3	0.72	46.2	14.3	0.72
Mean	68.3	30.4	1.35	68.3	30.4	1.34
Maximum	84.8	52.2	1.96	84.8	52.3	1.96
SD	6.7	6.6	0.22	6.7	6.6	0.22
Reg. method	PLS	PLS	PLS	PLS	PLS	PLS
Range (cm^{-1})	9002-3903	9002-3903	9002-3903.	9002-3903	9002-3903	9002-3903
Step (cm^{-1})	8	8	8	8	8	8
Predictor preprocess	2D(3,19); MC	2D(3,19); MC	2D(3,15); MC	SNV; Det; 2D(3,19); MC	SNV; Det; 2D(3,19); MC	2D(3,15); MC
Cross-validation	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)
LV	5	5	6	3	3	5
RMSEC	1.4	1.4	0.15	3.7	3.6	0.16
R^2C	0.95	0.95	0.51	0.69	0.71	0.46
RPDC	4.7	4.6	1.4	1.8	1.8	1.4
RMSECV	1.5	1.5	0.16	3.8	3.6	0.17
R^2CV	0.95	0.95	0.44	0.68	0.69	0.37
RPDCV	4.5	4.4	1.3	1.8	1.8	1.3
SEL prediction	1.0	1.0	0.06	0.5	0.5	0.04
Validation						
N	49	49	49	49	49	49
Outliers	0	0	0	0	0	0
Minimum	61.4	18.8	0.74	61.4	18.8	0.74
Mean	70.8	28.2	0.98	70.8	28.2	0.98
Maximum	80.4	37.3	1.35	80.4	37.3	1.35
SD	4.7	4.6	0.14	4.7	4.6	0.14
RMSEP	1.2	1.1	0.34	2.8	2.7	0.38
R^2P	0.95	0.95	0.21	0.65	0.65	0.54
RPDP	4.5	4.4	1.1	1.7	1.7	1.5
Bias	-0.6	0.3	0.30	0.02	-0.4	0.37
SEP	1.1	1.1	0.14	2.8	2.7	0.1
Slope	1.03	1.04	0.45	0.99	1.00	0.81
Intercept	-1.7	-1.4	0.4	0.4	0.5	-0.1
Avg T^2	3.4	3.3	5.2	4.7	4.7	6.1
SEL prediction	0.7	0.7	0.05	0.5	0.5	0.04

Wet NIRS is NIRS measured on wet maize silages and dry NIRS is NIRS measured on dry maize silage powders. 2D: 2nd derivative, Avg T^2: average Hotelling's T^2, CM: crude matter, Det: detrend, LV: latent variables, MC: mean-center, N: sample number, PLS: partial least squares, R^2C/CV/P: coefficient of determination of calibration/cross-validation/validation, Rdm: random, RMSEC/CV/P: root mean square error of calibration/cross-validation/validation, RPDC/CV/P: ratio of standard error of prediction to sample standard deviation for calibration/cross-validation/prediction, SD: standard deviation, SEL: standard error of laboratory, SEP: standard error of prediction, SNV: standard normal variate.

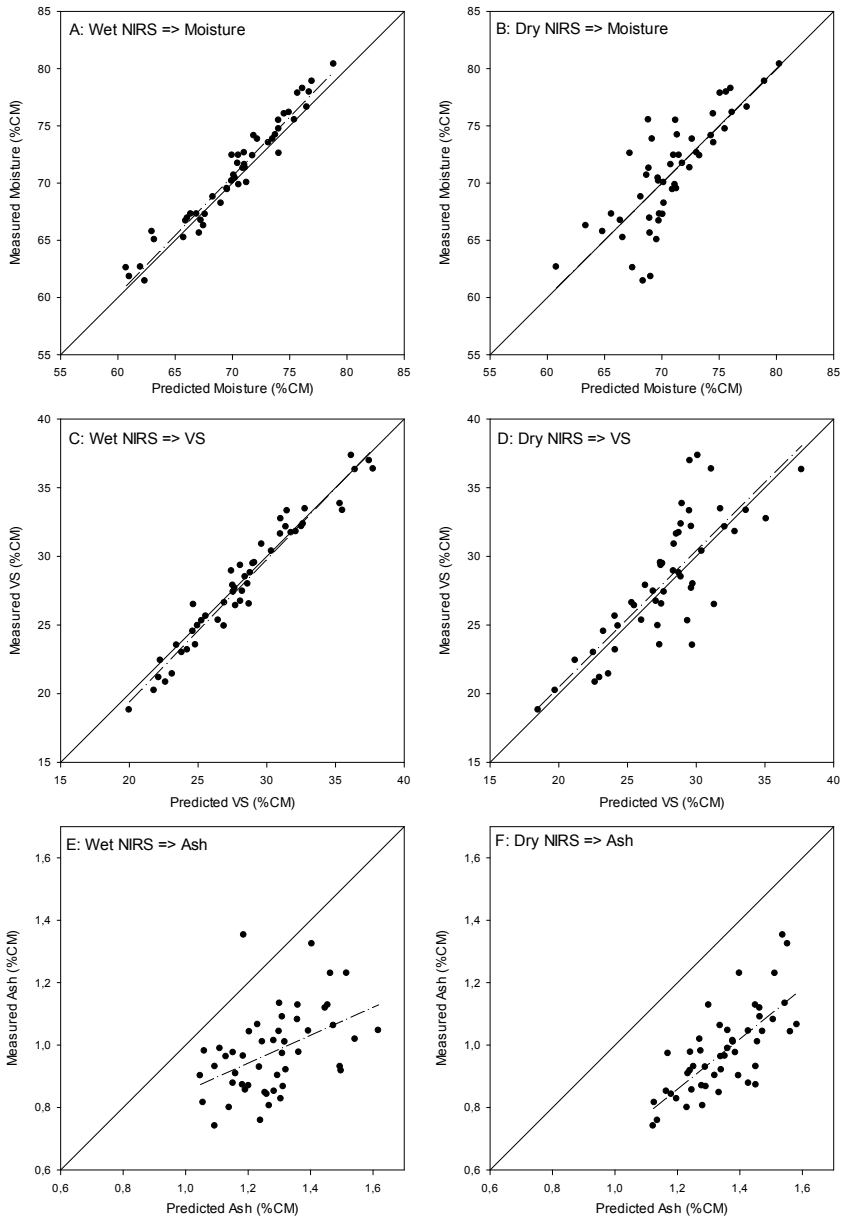

Figure 4.3. Measured and predicted moisture (A and B), volatile solids (VS, C and D) and ash (E and F) contents of wet maize silages with near infrared spectroscopy (NIRS). Wet NIRS (A, C and E) is NIRS measured on wet maize silages and dry NIRS (B, D and F) is NIRS measured on dry maize silage powders. Results of the validation set are presented.

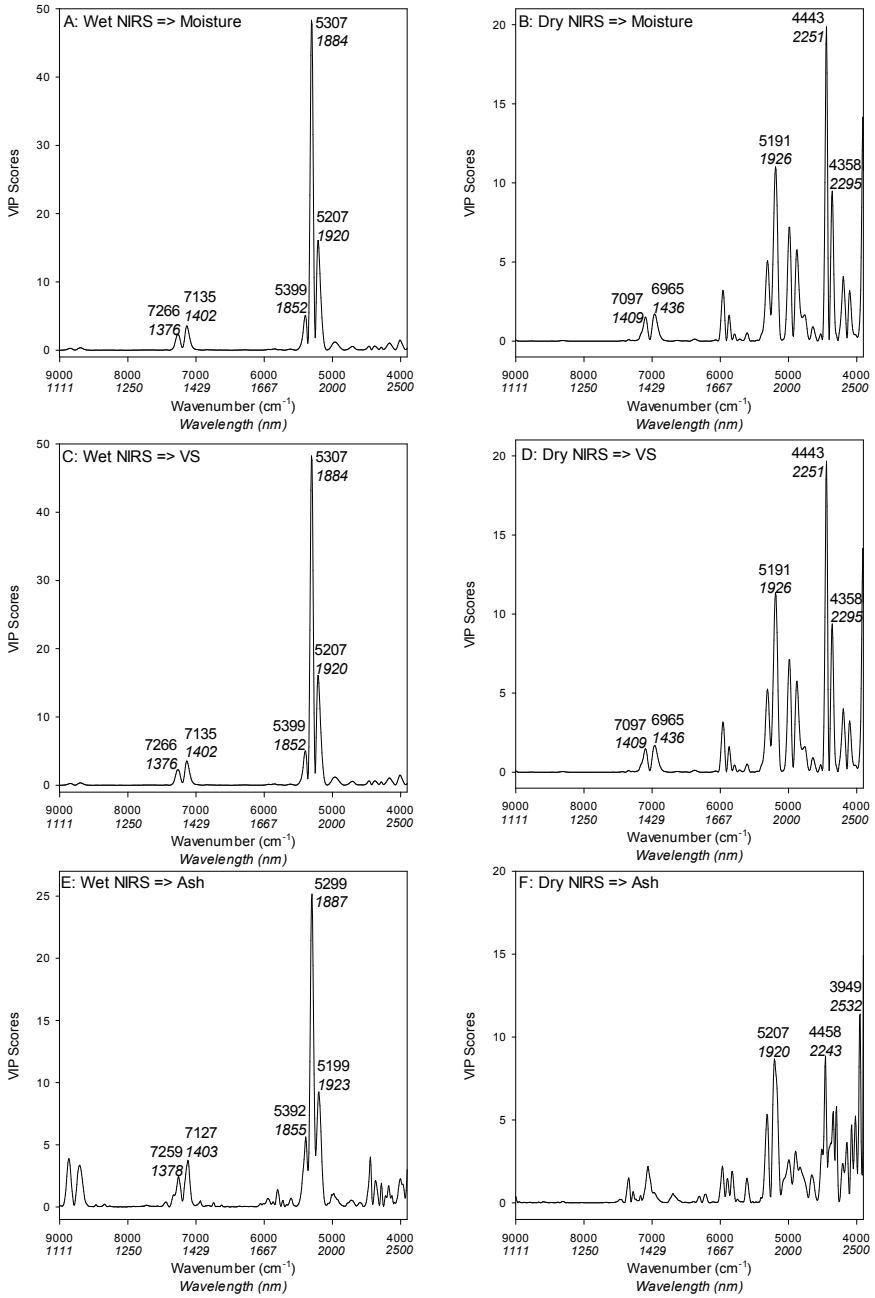

Figure 4.4. Variable importance in projection (VIP) scores of models that predict the moisture (A and B), the VS (C and D) or the ash (E and F) contents of wet maize silages. Predictors are near infrared spectra (NIRS) of wet maize silages (wet NIRS, A, C and E) or NIRS of dry maize silage powders (dry NIRS, B, D and F).

Prediction of biochemical parameters from dry NIRS

The hemicellulose, cellulose, ADL, elemental C, CP and fat contents were not measured for the samples included in the validation set (Table 4.1). The cross-validation results were thus used to assess the predictions. Table 4.4 and Table 4.5 present the results of the calibration and cross-validation of models that predict hemicellulose, cellulose, ADL contents and elemental C, CP and fat contents from wet NIRS and dry NIRS. The predicted and the measured values are shown in Figure 4.5 and Figure 4.6. The corresponding VIP scores of prediction models are shown in Figure 4.7 and Figure 4.8. No model was calculated for starch since this parameter was already measured from NIRS.

According to RPDCV (Table 4.4 and Table 4.5), Figure 4.5 and Figure 4.6, the predictions of cellulose, CP and fat were accurate with dry NIRS models (RPDCV of 3.2, 2.2 and 2.2 respectively), whereas hemicellulose, ADL and elemental C were less accurately predicted (RPDCV of 1.7, 1.5 and 1.3 respectively). The dry NIRS models predicting the cellulose, CP and fat contents can be used as screening tools for agro industry (Conzen, 2006).

The low R^2C for hemicellulose and ADL (0.67 and 0.58 respectively, Table 4.4) were expected according to their low R^2_{max} (0.82 and 0.77 respectively, Table 4.1). However, elemental C had a higher R^2_{max} than fat (0.90 and 0.88 respectively, Table 4.1) while it was less accurately predicted than fat (R^2C of 0.49 and 0.83 respectively, Table 4.5). Better prediction results were thus expected for elemental C than for fat. The determination of the R^2_{max} is thus valuable but prediction results with low accuracy can be obtained from the modelling procedure despite a high R^2_{max}.

Figure 4.7 shows similar VIP scores for the dry NIRS models predicting the content of the three fibre fractions. They are characterized by high values at 4435-4443 cm^{-1} and 4366 cm^{-1} (Figure 4.7). The region between 5307 cm^{-1} and 5176 cm^{-1} was also important for the prediction and not totally similar between the three dry NIRS models. Prediction models for hemicellulose, cellulose and ADL use similar spectral information. Since hemicellulose, cellulose and ADL were correlated in the dataset of the present study (Table 4.2), it can be hypothesized that prediction models made use of the same spectral information and the relationships between hemicellulose,

cellulose and ADL to predict their content in maize silages. Campo et al. (2013) reported better prediction results (RPD of 3.4-3.5) for the prediction of NDF and ADF. However, these two parameters are better correlated (r = 0.96) in Campo's study.

The highest VIP scores for dry NIRS model predicting the CP content are observed at 4335 cm^{-1}, 4443 cm^{-1} and 4605 cm^{-1} (Figure 4.8) CP is a biochemical fraction characterized by the presence of N atoms. Groups containing N-atoms absorb in a higher wawenumber range in the near infrared (Conzen, 2006). The VIP scores of the dry NIRS model predicting the CP content were not specifically related to ranges where groups containing N atoms absorb. Park et al. (2005b) reported better prediction results for the prediction of CP (R^2V of 0.91 and SEP of 0.29), however important predictors in the model were not detailed or explained.

The VIP scores of dry NIRS model predicting the fat content was high at 4343 cm^{-1} and 4435 cm^{-1} (Figure 4.8). These peaks are included in the spectral range of combination bands of aliphatic carbon absorbance (Conzen, 2006). The long chain fatty acid available in maizes (Jiménez et al., 2009), characterized by the succession of -CH_2 groups, might explain the importance of this spectral range in this model.

Table 4.4. Prediction models of cellulose, hemicellulose and acid detergent lignin (ADL) contents in dry maize silage powders with near infrared spectroscopy (NIRS).

Calibration and cross-validation						
Substrate	Dry maize silage powder					
Predictor	Wet NIRS			Dry NIRS		
Parameter	Hemicellulose	Cellulose	ADL	Hemicellulose	Cellulose	ADL
Unit	(%TS)	(%TS)	(%TS)	(%TS)	(%TS)	(%TS)
N	351	353	328	351	355	329
Outliers	2	0	6	4	0	7
Min	12.3	11.6	0.49	12.3	11.6	0.49
Mean	16.6	19.6	1.44	16.6	19.6	1.44
Max	24.9	36.1	2.83	24.9	36.1	2.83
SD	2.0	3.2	0.43	1.9	3.2	0.43
Reg. method	PLS	PLS	PLS	PLS	PLS	PLS
Range (cm^{-1})	9002-3903	9002-3903	9002-3903	9002-3903	9002-3903	9002-3903
Step (cm^{-1})	8	8	8	8	8	8
Predictor preprocess	2D(3,15); MC	2D(3,15); MC	2D(3,15); MC	SNV; Det; 2D(3,15); MC	2D(3,15); MC	SNV; Det; 2D(3,15); MC
Cross-validation	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)
LV	5	4	4	4	4	5
RMSEC	1.5	1.9	0.34	1.1	1.0	0.28
R^2C	0.40	0.64	0.36	0.67	0.91	0.58
RPDC	1.3	1.7	1.3	1.7	3.3	1.5
RMSECV	1.6	2.0	0.36	1.1	1.0	0.29
R^2CV	0.34	0.61	0.31	0.65	0.90	0.54
RPDCV	1.2	1.6	1.2	1.7	3.2	1.5
Slope	0.93	0.99	0.92	0.99	1.00	0.96
Intercept	1.1	0.24	0.11	0.20	0.08	0.05
SEL prediction	0.67	0.74	0.12	0.23	0.36	0.04

Wet NIRS is NIRS measured on wet maize silages and dry NIRS is NIRS measured on dry maize silage powders. 2D: 2nd derivative, Det: detrend, LVs: latent variables, MC: mean-center, N: sample number, PLS: partial least squares, R^2C/CV/P: coefficient of determination of calibration/cross-validation/validation, Rdm: random, RMSEC/CV/P: root mean square error of calibration/cross-validation/validation, RPDC/CV/P: ratio of standard error of prediction to sample standard deviation for calibration/cross-validation/prediction, SEL: standard error of laboratory, SEP: standard error of prediction, SNV: standard normal variate.

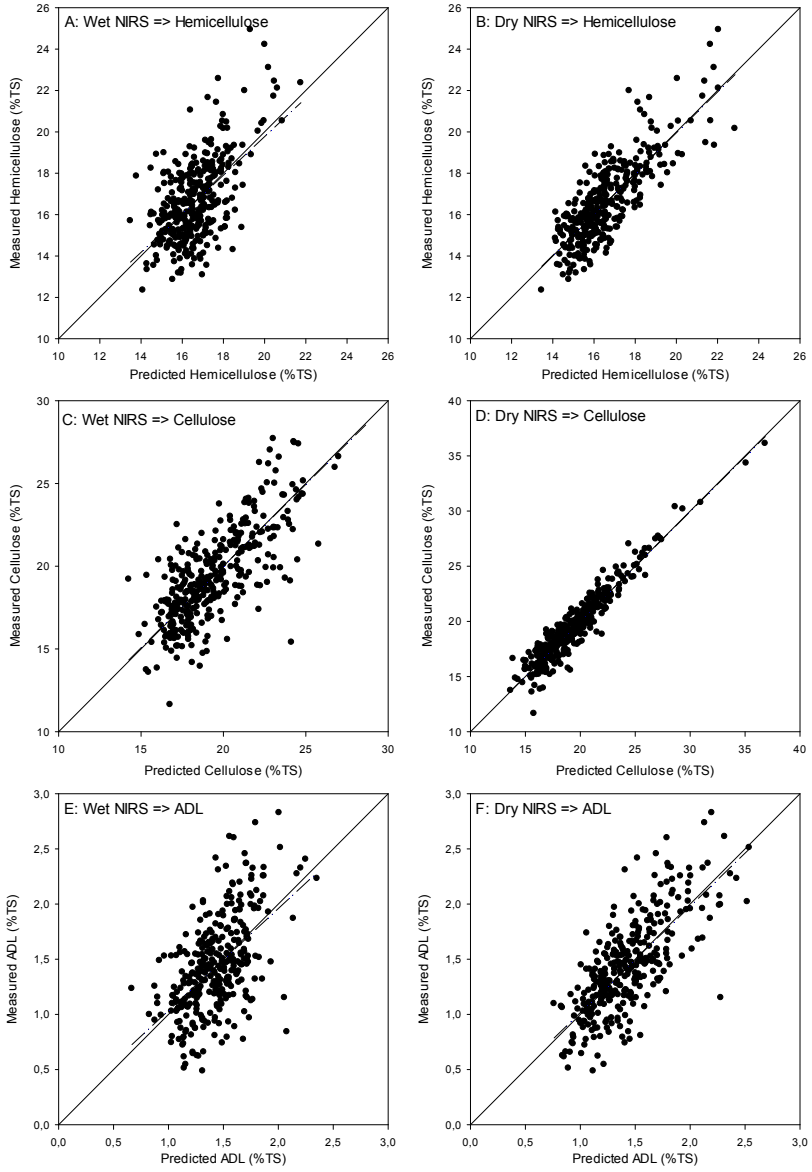

Figure 4.5. Measured and predicted hemicellulose (A and B), cellulose (C and D) and acid detergent lignin (ADL, E and F) contents of dry maize silage powders with near infrared spectroscopy (NIRS). Wet NIRS (A, C and E) is NIRS measured on wet maize silages and dry NIRS (B, D and F) is NIRS measured on dry maize silage powders. Results of the cross-validation are presented.

Table 4.5. Prediction models of crude proteins (CP), elemental C (C) and fat contents in dry maize silage powders with near infrared spectroscopy (NIRS).

Calibration and cross-validation						
Substrate	Dry maize silage powder					
Predictor	Wet NIRS			Dry NIRS		
Parameter	CP	C	Fat	CP	C	Fat
Unit	(%TS)	(%TS)	(%TS)	(%TS)	(%TS)	(%TS)
N	351	348	257	354	351	257
Outliers	0	3	5	0	3	5
Min	5.1	44.3	0.9	5.1	44.3	1.2
Mean	7.1	45.5	2.7	7.1	45.6	2.7
Max	10.3	47.0	3.9	10.3	47.0	4.2
SD	1.1	0.5	0.49	1.1	0.5	0.49
Reg. method	PLS	PLS	PLS	PLS	PLS	PLS
Range (cm^{-1})	9002-3903	9002-3903	9002-3903	9002-3903	9002-3903	9002-3903
Step (cm^{-1})	8	8	8	8	8	8
Predictor preprocess	2D(3,15); MC	2D(3,15); MC	2D(3,15); MC	SNV; Det; 2D(3,15); MC	SNV; Det; 2D(3,15); MC	SNV; Det; 2D(3,15); MC
Cross-validation	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)
LV	4	6	9	6	6	6
RMSEC	0.62	0.42	0.30	0.45	0.37	0.20
R^2C	0.66	0.38	0.62	0.81	0.49	0.83
RPDC	1.7	1.3	1.6	2.3	1.4	2.4
RMSECV	0.64	0.45	0.38	0.49	0.40	0.22
R^2CV	0.63	0.27	0.42	0.79	0.43	0.80
RPDCV	1.7	1.2	1.3	2.2	1.3	2.2
Slope	0.98	0.87	0.87	0.99	0.94	0.99
Intercept	0.12	6.14	0.35	0.09	2.6	0.03
SEL prediction	0.33	0.19	0.35	0.11	0.08	0.06

Wet NIRS is NIRS measured on wet maize silages and dry NIRS is NIRS measured on dry maize silage powders. 2D: 2nd derivative, Det: detrend, LVs: latent variables, MC: mean-center, N: sample number, PLS: partial least squares, R^2C/CV/P: coefficient of determination of calibration/cross-validation/validation, Rdm: random, RMSEC/CV/P: root mean square error of calibration/cross-validation/validation, RPDC/CV/P: ratio of standard error of prediction to sample standard deviation for calibration/cross-validation/prediction, SEL: standard error of laboratory, SEP: standard error of prediction, SNV: standard normal variate.

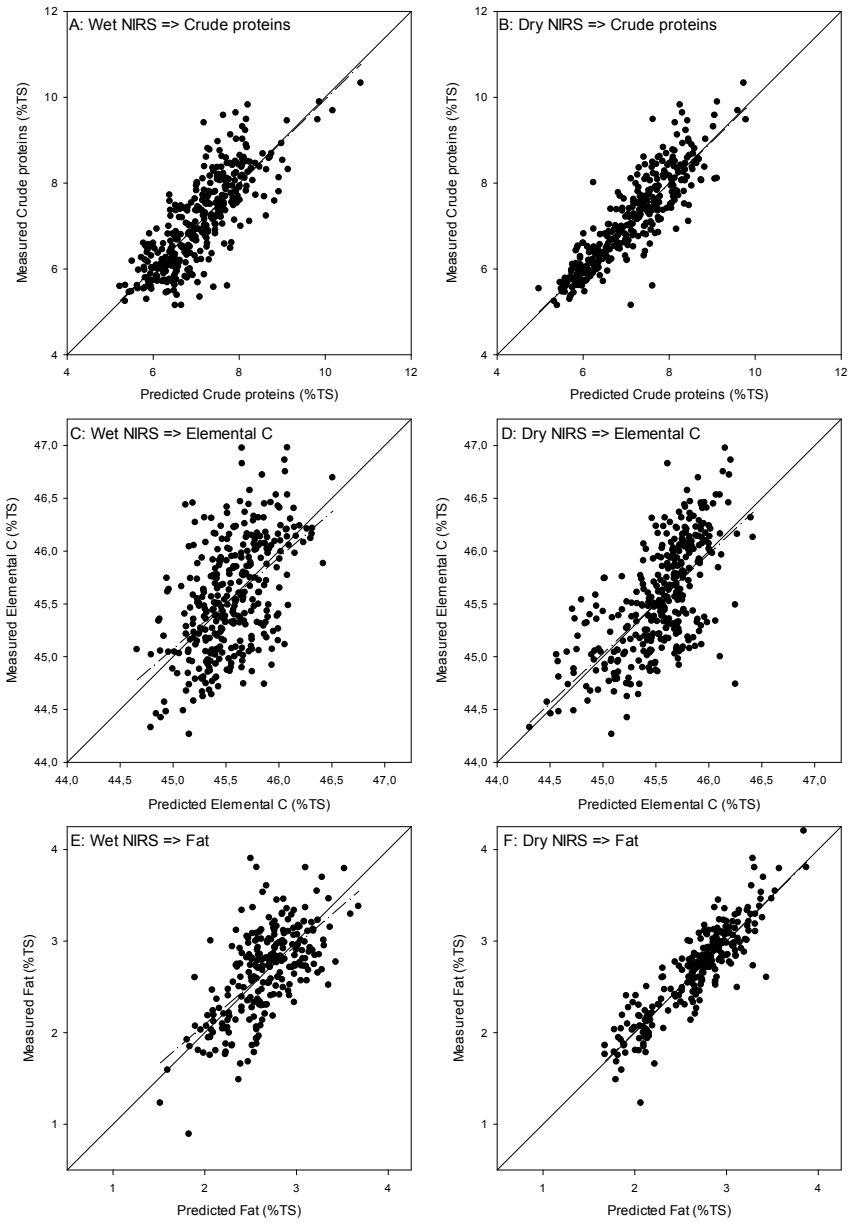

Figure 4.6. Measured and predicted crude proteins (A and B), elemental C (C and D) and fat (E and F) contents of dry maize silage powders with near infrared spectroscopy (NIRS). Wet NIRS (A, C and E) is NIRS measured on wet maize silages and dry NIRS (B, D and F) is NIRS measured on dry maize silage powders. Results of the cross-validation are presented.

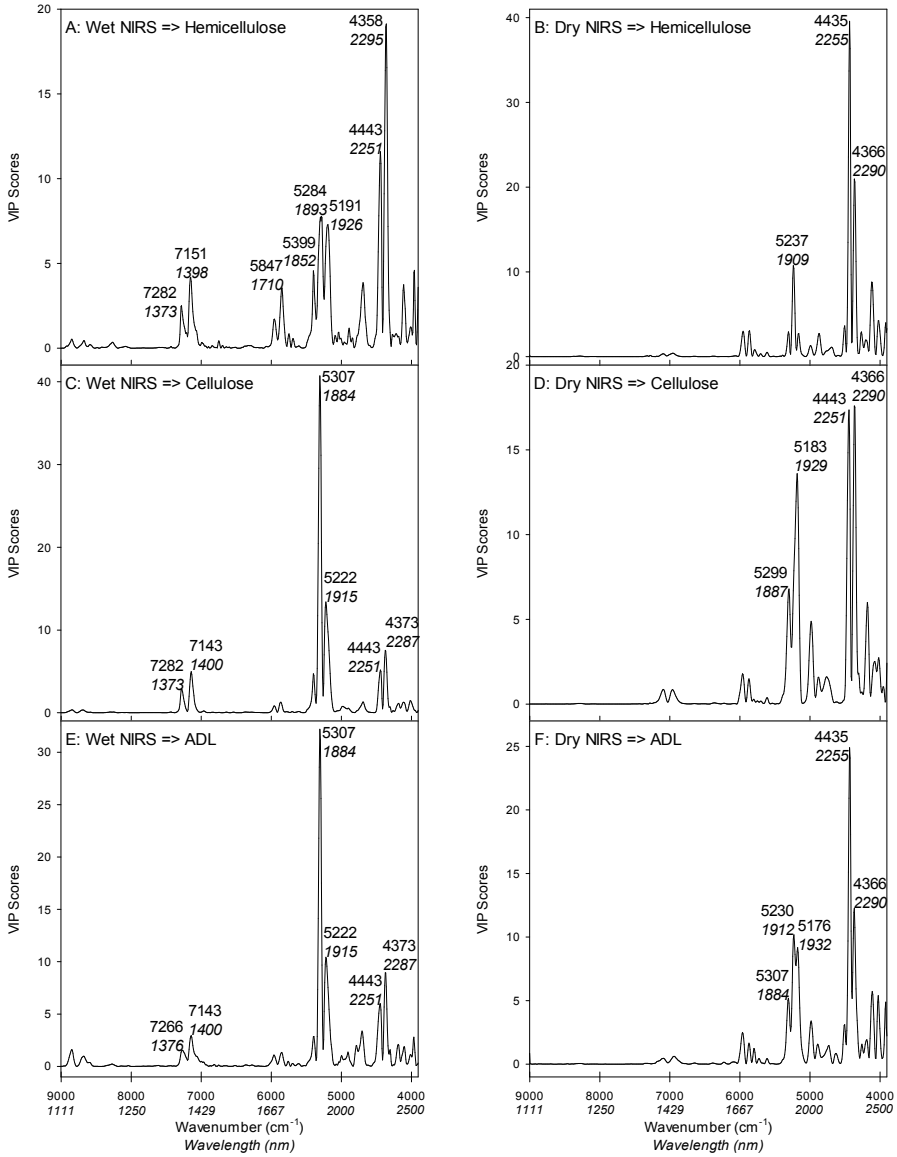

Figure 4.7. Variable importance in projection (VIP) scores of models that predict the hemicellulose (A and B), the cellulose (C and D) or the acid detergent lignin (ADL, E and F) contents of dry maize silage powders with near infrared spectroscopy (NIRS). Wet NIRS (A, C and E) is NIRS measured on wet maize silages and dry NIRS (B, D and F) is NIRS measured on dry maize silage powders.

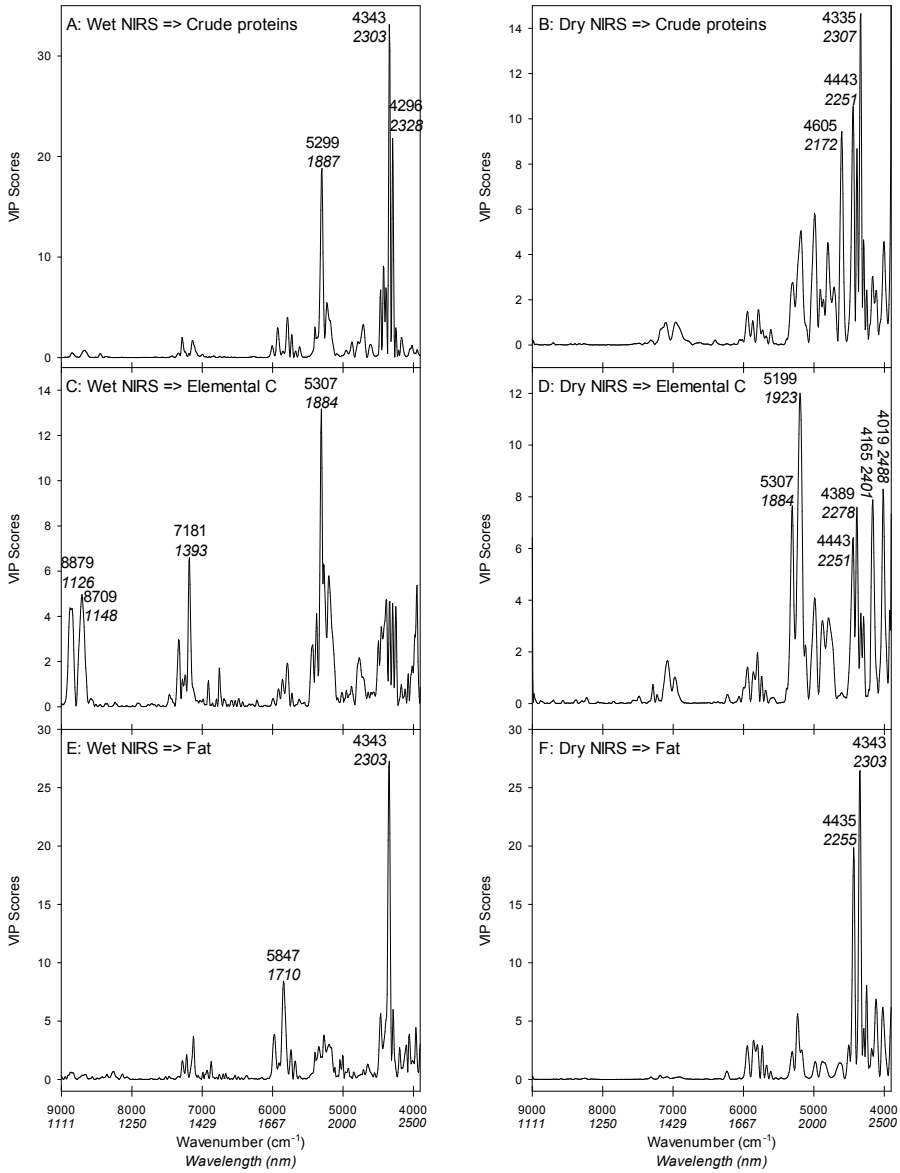

Figure 4.8. Variable importance in projection (VIP) scores of models that predict crude proteins (A and B), elemental C (C and D) or fat (E and F) of dry maize silage powders with near infrared spectroscopy (NIRS). Wet NIRS (A, C and E) is NIRS measured on wet maize silages and dry NIRS (B, D and F) is NIRS measured on dry maize silage powders.

Comparison of wet NIRS and dry NIRS

The moisture and VS contents of wet maize silages were better predicted with wet NIRS models (Table 4.3, RPDP of 4.4-4.5) than with dry NIRS models (Table 4.3, RPDP of 1.7). It should not be possible to directly measure the moisture content of wet maize silages from dry maize silage powders. Surprisingly, models based on dry NIRS were able to predict the moisture and VS contents of wet maize silages. Information related to the moisture and the VS contents of wet maize silages was present in dry maize silage powders. Indeed, the VS and the moisture contents were correlated or anticorrelated respectively with starch and fibres, especially the cellulose content (Table 4.2). Moreover high VIP scores were observed in the same spectral region (5191-5183, 4443-4435 and 4366-4358 cm^{-1}) for dry NIRS models that predict the VS or moisture contents (Figure 4.4) and the cellulose content (Figure 4.7). Therefore, the prediction of the moisture and VS contents of wet maize silages is possible with dry NIRS because of the correlations of these parameters and biochemical parameters such as cellulose of dry maize silage powders. Liu and Han (2006) already observed this result and explained it similarly by correlations between the VS content and the biochemical composition of dry maize silage powders.

Following the same reasoning, similar VIP scores were observed for wet NIRS models predicting moisture or VS contents (Figure 4.4) and cellulose content (Figure 4.7). The prediction of the cellulose content from wet NIRS model can be attributed to the correlation between the cellulose content and the moisture or VS contents. Wet NIRS can therefore be used as a first screening tool to predict the cellulose content in wet maize silages, without time-consuming physical pretreatment (Park et al., 2005).

Dry NIRS models predicted better most of the biochemical compounds (cellulose, hemicellulose, ADL, CP and fat) than wet NIRS models (Table 4.4, Table 4.5, Figure 4.5 and Figure 4.6). Wet maize silages have a moisture content of 68.3 %CM on average (Table 4.1). This amount of water absorbs NIR photons and dilutes the information in the spectra. Indeed, variability of the samples around the regression lines was higher for the wet NIR models than for the dry NIR models when predicting organic biochemical compounds (Figure 4.5 and Figure 4.6). Better predictions from wet NIRS could be obtained through the improvement of the

accuracy of the spectral measurement. Such an improvement can be realized by increasing the number of spectral replicates to obtain the necessary number of recordings needed to achieve a representative spectra of a wet maize silage (Jacobi et al., 2011). The use of shorter wavelengths (780-1100 nm, corresponding to 12821-9091 cm^{-1}) in the Herschel infrared region was shown to improve calibration statistics (Cozzolino et al., 2006). However this spectral range was not measurable with the NIR spectrometer used in the present paper.

A grounding step was realized after the drying of wet maize silages to obtain the dry maize silage powders. It allowed the reduction of particle size and a higher homogeneity of samples when presented to the spectrometer. A higher homogeneity could also improve the spectral measurement. The influence of the sample preparation on the prediction of dry matter, pH and short chain organic acids was shown by Park (Park et al., 2005). Indeed in Park's study, dry matter was better predicted with spectra measured on samples that were frozen in liquid nitrogen and ground ($R^2V = 0.81$) than with intact fresh samples ($R^2V = 0.70$). Both drying and grounding improved the prediction of organic biochemical parameters using NIRS.

4. Conclusion

Due to a very low content of ash, maize silage can be considered as a bi-component product made of moisture and VS. Starch and fibres are the most abundant fractions in maize silages and are related to the VS content. Before carrying on modelling procedure, the determination of the R^2_{max} is advised since it is informative on the feasibility of a prediction model while avoiding inadequate and time-consuming work.

The prediction of the moisture and VS contents from wet NIR spectra is fully suitable for routine measurement. Models that predict the biochemical composition of maize silages using NIRS of wet maize silages are feasible but are less accurate than those using NIRS on dry and ground material. Models based on NIRS use correlations between biochemical compounds, such as VS and moisture or VS and cellulose, to predict the concentration of these compounds. Importance of spectral contributions should be highlighted when presenting prediction models to improve and orientate further research.

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Highlights of Chapter 4

- Maize silage can be considered as a bi-component product made of moisture and VS because of its low ash content.
- The VS content is mainly composed of starch and fibres.
- The moisture and VS contents can be predicted with models based on wet NIRS.
- Models based on dry NIRS are more accurate than models based on wet NIRS to predict the biochemical composition of totals solids of maize silages.
- The determination of the R^2_{max} is advised before carrying on modelling procedure.
- The VIP scores should be also reported with prediction model statistics and graphs presenting measured vs. predicted values.

Chapter 5

Prediction of the biochemical methane potential of wet maize silages with biochemical composition or near infrared spectroscopy

In Chapter 3, silages of maize cropped under various agro-climatic conditions have been produced and analysed for their BMP as crude wet silages. The VS content was shown to influence the BMP_{CM} of wet maize silages. In Chapter 4, the biochemical composition of the VS content of maize silages was analysed, and models based on NIRS were shown to be able to predict this composition. Important spectral region for the predictions have been highlighted, especially the range corresponding to water absorbance. In a following step, we will evaluate **what is the ability of models based on the biochemical composition or NIRS to predict the BMP of crude maize silages.**

In the present chapter (Chapter 5), the effect of the biochemical composition on the BMP of wet maize silages is assessed. Prediction of BMP from models based on NIRS is also investigated and the important spectral regions for such predictions are highlighted.

Abstract

Measuring the biochemical methane potential (BMP) is a time-consuming assay (at least one month) and requires dedicated scientific instruments. Therefore, the ability to predict the BMP of wet maize silages from the biochemical composition or from near infrared spectroscopy (NIRS) data was investigated. Maize was cropped under various agro-climatic conditions in Belgium and in Luxembourg and silaged. The BMP and the biochemical composition of these maize silages were characterised. A model using the total solids (TS) content can predict the BMP of maize silages on a crude matter basis (BMP_{CM}) with the same accuracy (standard error of prediction of 5 $mL.gCM^{-1}$, equivalent to 4.5% of the mean BMP_{CM}) and faster than a model based on the volatile solids (VS) content. NIR spectra were measured on wet maize silages and their respective dried and ground powders. NIR spectra of wet maize silages can be used in a model to predict the BMP_{CM} of wet maize silages with good accuracy (standard error of prediction of 7 $mL.gCM^{-1}$ equivalent to 5.5% of the mean BMP_{CM}) and faster than the model based on the TS content. This can be explained by the ability of NIRS to efficiently predict the VS content of wet silages, and the direct relationship between the VS content, the TS content and the BMP. The BMP_{CM} of a sample predicted with a model that was calibrated with samples from the same country was more accurate owing to a bias diminution. The BMP on volatile solid basis (BMP_{VS}) was not accurately predicted because the repeatability of the BMP assay (standard error of laboratory of 22 $mL.gVS^{-1}$, equivalent to 5.3% of the mean BMP_{VS}) remained insufficient as compared to the low variability of the BMP_{VS} of maize silages (standard deviation of 41 $mL.gVS^{-1}$).

Keywords: biochemical methane potential, near infrared spectroscopy, prediction, maize, silage, energy crops, biogas, biomethane, bioenergy.

1. Introduction

Anaerobic digestion is seen as an environmental friendly and competitive way to produce renewable energy, with a contribution to the reduction of greenhouse gases emission (Weiland, 2009). A wide variety of substrate can be converted into energy through biomethanation (Raposo et al., 2012). Energy crops are particularly investigated as a complement for agricultural biogas plants and various plant species were already assessed (Bauer et al., 2009, Mayer, 2014).

In Europe, maize, processed and stored as silage, is the main energy crop used in biomethanation thanks to its high biomethane yield. Such a biomethane yield results from both its high biomass yield (amount of biomass per ha and year) and its high biochemical methane potential (BMP, methane produced per amount of biomass) as compared to other plant species. However, BMP of maize silages are very variable (Mayer et al., 2014) and such variability affects the biomethane yield. The characterization of BMP is thus important in order to manage properly anaerobic digester feedstock.

The reference method to measure the BMP consists in a batch anaerobic digestion in a laboratory digester. However, such a method is time-consuming (up to 60 days) and tedious to implement because of the requirement of dedicated working space and laboratory instruments. Fast analytical techniques, such as biochemical composition analysis or spectroscopy, were reviewed to quickly obtain BMP values (Lesteur et al., 2010).

Since a long time ago, biochemical composition has been considered to calculate the BMP of various energy crops (Buswell & Mueller, 1952). Such an approach gave successful results for various biomasses (Nallathambi Gunaseelan, 2009, Nallathambi Gunaseelan, 2007, Amon et al., 2007). However, analytical methods that determine the biochemical composition still require work and time, produce wastes and are destructive for biomass.

Fast analytical technics were developed to speed up the analysis of the biochemical composition of feed and food crops. Among them, near infrared spectroscopy (NIRS) appeared to be very successful and is now commonly used to predict parameters such as the content in dry matter, fibers, crude proteins, fat or starch of various feed and food products (Chapter 3).

Since NIRS is widely used to successfully characterize agricultural biomass in term of composition, such an analytical technic appears to be a promising tool to predict BMP of organic materials (Lesteur et al., 2011, Doublet et al., 2013), including energy crops (Kandel et al., 2013; Raju et al., 2011). More specifically, BMP of dry maize silage powders has been predicted in laboratory with its biochemical composition and with NIRS (Grieder et al., 2011). BMP of wet maize silages has also been determined on-line with NIRS, in a biogas plant (Jacobi et al., 2012).

The aim of this article is to assess the ability of both biochemical composition and NIRS to predict the BMP of crude wet maize silages in laboratory. The accuracy and the required time of both methods were compared. The most important predictors of BMP among the biochemical parameters on one hand and among the spectral data of NIRS on the other hand, were investigated.

2. Material and methods

2.1. Maize production

The production of the maize silages was described in details in a previous study (Mayer et al., 2014). Briefly, maize was cropped in Luxembourg and in Belgium from 2007 to 2009 in 13 environments (location x year). Different varieties with various maturity classes (FAO index from 220 to 340 mainly) were sown and harvested at different dates. A total of 379 samples was collected.

The maize trials initiated over the 2007-2009 period were continued the following years. A specific trial involving four maize varieties with various maturity indexes (FAO index from 240 to 340) in a randomized complete block design were continued in 2010 and in 2011, in Useldange and in Nagem (Luxembourg), respectively. An additional sample harvested in Useldange (Luxembourg) in 2012 (variety Subito) was also included. A total of 49 samples was collected over the 2010-2012 period (Chapter 3).

Fresh maize samples were ensiled in plastic bags under vacuum and stored at room temperature until further laboratory analysis.

2.2. Biochemical measurements

The moisture, the volatile solids (VS) and the ash contents of the wet maize silages were measured in triplicates. Total solids (TS) content consisted in the sum of the ash and the volatile solid contents (Chapter 3).

Subsamples were taken out of the maize silages, dried in an oven at 70°C during 48h, then ground through a 1-mm mesh in a centrifugal mill (Cyclotec, Foss). The obtained dry maize silage powders were used for the characterization of the biochemical composition.

Hemicellulose, cellulose, acid detergent lignin (ADL), fat, crude proteins (CP), elemental C and N and starch were measured as described by Mayer et al. (Chapter 3). In brief, hemicellulose and cellulose were measured with an automatic instrument (Ankom 2000, Ankom Technology) after Van Soest digestions (Ankom, 2006a; Ankom, 2006b). The remaining matter was digested in beakers containing 72% sulfuric acid solution to obtain the ADL content (Ankom, 2005). Fat was gravimetrically quantified after a Soxhlet extraction and solvent evaporation (AOAC International, 2006). Elemental C and elemental N were measured with an elemental analyser (TruSpec, LECO). The CP content was obtained by multiplying the elemental N content by 6.25. Starch was predicted with a model based on near infrared spectra of dry maize silage powders in the *Centre walloon de Recherches agronomiques* (Gembloux, Belgium).

2.3. BMP measurements

The biochemical methane potential for the maize silage samples of 2007-2009 was assessed in a previous paper (Mayer et al., 2014a). The new set of maize silages harvested in 2010, 2011 and 2012 was also measured according to this method, with modifications (automation and condensing of the water vapor in the biogas) as described by Mayer et al. (2014b). In brief, BMP assays were carried out in 2L total capacity digesters. Inoculum and wet maize samples were incubated in the digesters kept at 37°C. The biogas was collected in 10L gas bags and regularly analysed for its volume and composition (methane and carbon dioxide). The biomethane measurements were normalised (273 K, 1013 hPa) and cumulated at the end of the assay to obtain the BMP per unit of crude matter (BMP_{CM}). The BMP was then

expressed per unit of volatile solids (BMP_{VS}) using the VS content of wet maize silages. The biomethane production of the inoculum was substracted from those of samples. The degradation activity of the inoculum was validated by digesting microcrystalline cellulose. All batch digestions were carried out in triplicate.

2.4. NIRS measurements

Near infrared spectra were measured on wet maize silages and on dry maize silage powders (Chapter 3). Near infrared (NIR) spectra were acquired on both wet maize silages (wet NIRS) and dry maize silage powders (dry NIRS). NIR spectra were measured in an air-conditioned laboratory with a Bruker MPA spectrometer (Bruker Optik GmbH) operated with OPUS 6.5. The spectrometer was equipped with an integrating sphere for reflectance measurements. Before each measurement session, the spectrometer was validated according to the procedure installed in the software (PQ test). A background measurement was performed before the first measurement and each hour of the measurement session.

For the NIR spectrum acquisition, a hard coated aluminium sample cup of 97mm diameter with a water free quartz window (IN 312-S, Bruker) was filled with the sample, before being placed on a rotating sample cup holder (IN 312/C, Bruker). The window of the spectrometer was not aligned with the centre of the circular sample cup in order to scan a representative heterogeneous surface of 38 cm^2 during the rotation of the cup. Absorbance spectra were collected in the 3594-9989 cm^{-1} range with a resolution of 16 cm^{-1}. The measured NIR spectrum of a sample was the result of the average of 32 spectra automatically collected during the rotation of the sample cup. For each sample, three NIR acquisitions were realized after emptying, cleaning and repacking the sample cup, to obtain three NIR spectra corresponding to one single sample.

2.5. Data treatment and chemometrics

A first set, called the calibration set, was used to create and assess models with a cross-validation, and a second set, called the validation set, was used to test the models on new samples without any influence of the calibration procedure. Maize silages harvested in 2007, 2008 and 2009 were used in the calibration set. Samples

cropped in 2010, 2011 and 2012 were included in the validation set. Descriptive statistics were calculated with SPSS, version 19 (SPSS Inc.).

Before any model calculation, the feasibility of developing a prediction model was assessed according to Dardenne (2010). The standard error of laboratory (SEL) of BMP_{CM} and BMP_{VS} was calculated according to:

$$SEL = \sqrt{\frac{\sum s}{N}}$$

with *s*, the variance of the replicate analysis for one sample and *N* the number of samples in the dataset.

The maximum coefficient of determination of any calibration (R^2_{max}) was calculated using:

$$R^2_{max} = \frac{SD^2 - SEL^2}{SD^2}$$

with *SD*, the standard deviation of the dataset for the reference value.

Univariate regressions were calculated with Excel 2010 and multivariate data analysis was carried out with Matlab, version R2012a (MathWorks) and PLS_Toolbox, version 7.3.1 (Eigenvector Research, Inc.).

The triplicate NIR spectra of a sample were averaged to obtain one single spectrum per sample. Triplicate and average spectra were plotted to check outliers before being used in the model calculation as predictors. In each model calculation and for each reference parameter, the calibration set and the validation set were described with the number of total available samples, the number of outliers, the minimum value, the maximum value, the mean value and the standard deviation (SD). The regression methods used were the simple linear regression for univariate models and the partial least squares (PLS) for multivariate models.

Preprocessing methods included autoscaling (AS) for biochemical data before being used in a multivariate calibration procedure. For the spectral data, mean centering (MC), autoscaling (AS), standard normal variate (SNV) or detrend (Det) were investigated as preprocessing methods. Derivatives were also assessed and abbreviated as xD(y;z), with x the derivative order, y the polynomial order and z the filter width. The excluded spectral data were used in the derivative calculations, but not in the subsequent model.

A cross-validation was used during the calibration procedure to find the optimal number of latent variables (LV) for multivariate models. The cross-validation consisted in the random split of the calibration set in 10 segments, with the same number of samples in each segment. The calibration model was then calculated with all the samples from 9 segments. Using this calibration model, the value of the parameter was predicted for each sample of the 10th segment. This procedure was repeated 10 times to calculate the statistics of the model.

The root mean square error (RMSE), the coefficient of determination (R^2) and the ratio of standard error of prediction to sample standard deviation (RPD) was calculated for the calibration (RMSEC, R^2C, RPDC), cross-validation (RMSECV, R^2CV, RPDCV) and validation (RMSEP, R^2P, RPDP).

The RMSE was calculated according to:

$$RMSE = \sqrt{\frac{\sum(y_m - y_p)^2}{N}}$$

with y_m the measured values, y_p the predicted values and N the number of samples in the dataset.

The R^2 was calculated according to:

$$R^2 = \left(\frac{\sum(\widehat{y_m} - \overline{y_m})^2}{\sum(y_m - \overline{y_m})^2}\right)$$

with y_m the measured values, $\widehat{y_m}$ the estimated y_m values given by the regression line and $\overline{y_m}$ the mean y_m value.

The RPD, which is a more discriminant value than the R^2 value, was calculated according to:

$$RPD = \frac{1}{\sqrt{1 - R^2}}$$

For the validation set, the bias, corresponding to the systematic averaged deviation between the reference and the predicted values (Conzen, 2006), was calculated according to:

$$Bias = \frac{1}{N}\sum(y_m - y_p)$$

The standard error of prediction (SEP), which is the RMSEP corrected for the bias, was calculated according to:

$$SEP = \sqrt{\frac{1}{N-1}\sum\left(y_m - y_p - bias\right)^2}$$

The intercept and the slope were calculated for the linear regression between the predicted values (x-axis) and the measured values (y-axis) for the validation or the cross-validation data. The best model was chosen and presented in the paper according to the lowest RMSECV, the lowest number of LV and the highest R^2CV and RPDCV.

The variable importance in projection (VIP) scores give a summary of the importance of a predictor for both predictors and predicted parameter. A variable with a VIP score close to or greater than 1 can be considered as important in a given model (Wold et al., 2001). The VIP score for the *j*-th variable was calculated according to:

$$VIP_j = \sqrt{p\frac{\sum_{k=1}^{h}\left(b_k^2 t_k^t t_k \left(\frac{w_{jk}}{\|w_k\|}\right)^2\right)}{\sum_{k=1}^{h} b_k^2 t_k^t t_k}}$$

with *p* the number of predictors, *h* the number of LV, *k* the *k*-th LV, *b* the regression coefficient of the score matrix, *t* the column vector of the score matrix, *w* the column vector of the weight matrix (Chong & Jun, 2005).

The SEL of prediction for calibration sets and validation sets was calculated by applying the model to spectra replicates in order to obtain the predicted values.

3. Results and discussion

3.1. Prediction of BMP from biomass composition

The BMP_{CM} and the BMP_{VS} of crude wet maize silages measured for the calibration and the validation sets are presented in Figure 5.1 and Table 5.1. The ranges of BMP_{CM} and BMP_{VS} of maize harvested in 2010 and 2011 (74-147 $mL.gCM^{-1}$ and 362-449 $mL.gVS^{-1}$ respectively) were fully included in the ranges of BMP measured on maize harvested from 2007 to 2009 (39-201 $mL.gCM^{-1}$ and 276-557 $mL.gVS^{-1}$, respectively). The validation set was suitable to assess the models predicting the BMP (Conzen, 2006).

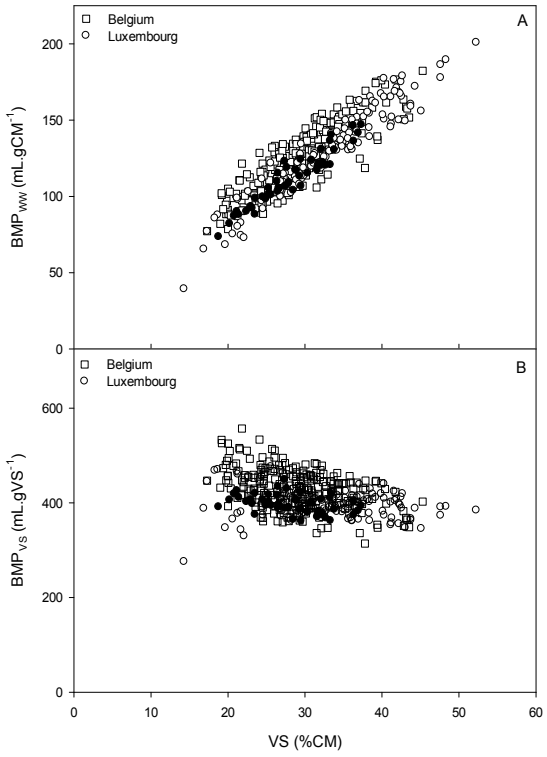

Figure 5.1. Influence of the volatile solids (VS) content of wet maize silages on the biochemical methane potential (BMP) on a crude matter basis (BMP_{CM}, A) and on a VS basis (BMP_{VS}, B). The calibration set is presented with open symbols and the validation set with closed symbols. Samples harvested in Belgium are presented as squares and samples harvested in Luxembourg are presented as circles.

Table 5.1. Biochemical methane potential (BMP) of wet maize silages for the calibration set and the validation set.

Substrate	Wet maize silage	
Parameter	BMP_{CM}	BMP_{VS}
Unit	$mL.gCM^{-1}$	$mL.gVS^{-1}$
Calibration set		
N	364	363
Minimum	39	276
Maximum	201	557
Range	161	281
Mean	126	418
SD	24.8	41.4
RSD (%)	19.7	9.9
SEL	5.3	22.1
RSEL (%)	4.2	5.3
SEL/SD (%)	21.2	53.4
R^2_{max}	0.96	0.72
Validation set		
N	49	49
Minimum	74	362
Maximum	147	449
Range	73	87
Mean	112	397
SD	17.1	19.6
RSD (%)	15.4	4.9
SEL	3.8	15.2
RSEL (%)	3.4	3.8
SEL/SD (%)	22.3	77.5

BMP are expressed on a crude matter basis (BMP_{CM}) and on a volatile solids basis (BMP_{VS}). N: number of samples, SD: standard deviation, SEL: standard error of laboratory, RSD: relative standard deviation, RSEL: relative standard error of laboratory, R^2_{max}: maximum coefficient of determination.

The R^2_{max} was 0.96 for BMP_{CM} and 0.72 for BMP_{VS} prediction (Table 5.1). The prediction of the BMP_{CM} of wet maize silages was expected to be more accurate than the prediction of the BMP_{VS}.

The prediction of BMP of wet maize silages was first tested with biochemical parameters (Table 5.2, Figure 5.2). Different models using either the TS content

only, or the VS content only, or the detailed biochemical composition (VS, ash, hemicellulose, cellulose, ADL, fat, C and CP) were investigated to predict the BMP_{CM} and BMP_{VS} of wet maize silages. Prediction models are identified below as TS models, VS models and biochemistry models, respectively.

The prediction of the validation set with the VS model showed a RPDP of 3.2 and a SEP of 5.3 $mL.gCM^{-1}$ (Table 5.2). Moreover, the SEP was equal to the SEL (Table 5.1, 5.3 $mL.gCM^{-1}$). The VS content was an accurate predictor of the BMP_{CM} of wet maize silages. Such good result was explained by the low variability in CH_4 content in the biogas produced by maize silages and a low variability of the anaerobic digestibility between the cropped maize (Mayer et al., 2014a).

Considering the very low amount of ash in maize silages (Chapter 3, Table 5.3, 1.4%CM on average) and the additional 24 hours required to measure this amount in order to obtain the VS content, a model based on the TS content of wet maize silages was calculated to predict the BMP_{CM} (Table 5.2). The TS model calibrated with maize samples harvested in Luxembourg and in Belgium showed similar results as the VS model (RPDP: 3.3, SEP: 5.2 $mL.gCM^{-1}$). The BMP_{CM} of wet maize silages can be predicted faster using the TS content rather than the VS content of wet maize silages, while accuracy is not negatively affected.

Since the BMP have been shown to be affected by the biochemical composition of the biomass, the prediction of BMP_{CM} of wet maize silages was expected to be more accurate with additional information from the biochemical composition of the maize silages. Table 5.3 presents the correlation coefficients between the BMP and the biochemical parameters used in the biochemistry model. After VS (r = 0.90), the BMP_{CM} had the highest correlation coefficients with the starch (r = 0.74) and cellulose (r = -0.67) contents (Table 5.3). Such relationships can be explained by the correlation coefficients measured between the VS and starch contents (r = 0.66) and between the VS and cellulose contents (r = -0.71) (Chapter 3, Table 5.2). Since the BMP_{CM} was highly correlated with the VS content, the BMP_{CM} was consequently (anti)correlated with the cellulose and the starch contents. The absolute values of correlation coefficients of other biochemical parameters with BMP_{CM} were lower than 0.5. The VS, starch and cellulose contents seemed to be the most significant parameters for the prediction of BMP_{CM}.

Table 5.2. Prediction models of the biochemical methane potential on a crude matter basis (BMP_{CM}) of wet maize silages with the volatile solids content or the total solids content or the biochemical composition.

Substrate	Wet maize silage				
Parameter	BMP_{CM}				
Unit	$mL.gCM^{-1}$				
Calibration and cross validation					
Harvest country	Luxembourg & Belgium			Luxembourg	
Predictor	VS (%CM)	TS (%CM)	Biochemistry	VS (%CM)	TS (%CM)
N	363	363	247	121	121
Outliers	1	1	0	1	1
Min	65	65	65	65	65
Mean	126	126	129	132	132
Max	201	201	201	201	201
SD	24	24	24.6	28	28
Predictor preprocess	-	-	AS	-	-
Reg. method	SR	SR	PLS (4LV)	SR	SR
RMSEC	10.6	10.5	10.1	8.2	8.1
R^2C	0.81	0.81	0.83	0.92	0.92
RPDC	2.3	2.3	2.4	3.4	3.5
SEL prediction	3.0	2.9	-	3.1	3.0
Cross-validation	-	-	Rdm(10;10)	-	-
RMSECV	-	-	10.5	-	-
R^2CV	-	-	0.82	-	-
RPDCV	-	-	2.3	-	-
Validation					
N	49	49	-	49	49
Outliers	0	0	-	0	0
Min	74	74	-	74	74
Mean	112	112	-	112	112
Max	147	147	-	147	147
SD	17	17	-	17	17
RMSEP	8.9	8.0	-	5.4	5.2
R^2P	0.91	0.91	-	0.91	0.91
RPDP	3.2	3.3	-	3.2	3.3
Bias	7.2	6.1	-	1.1	0.5
SEP	5.3	5.2	-	5.3	5.2
Intercept	-13.4	-12.2	-	3.7	4.2
Slope	1.05	1.05	-	0.96	0.96
SEL prediction	2.0	2.0	-	2.2	2.1

Biochemistry includes VS, ash, cellulose, hemicellulose, acid detergent lignin, crude proteins, elemental C, fat and starch. 2D: 2nd derivative, CM: crude matter, LV: latent variables, MC: mean-center, N: sample number, PLS: partial least squares, R^2C/CV/V: coefficient of determination of calibration/cross-validation/validation, RMSEC/CV/V: root mean square error of calibration/cross-validation/validation, RPDC/CV/P: ratio of standard error of prediction to sample standard deviation for calibration/cross-validation/prediction, SEL: standard error of laboratory, SEP: standard error of prediction, SR: simple regression,VS: volatile solids.

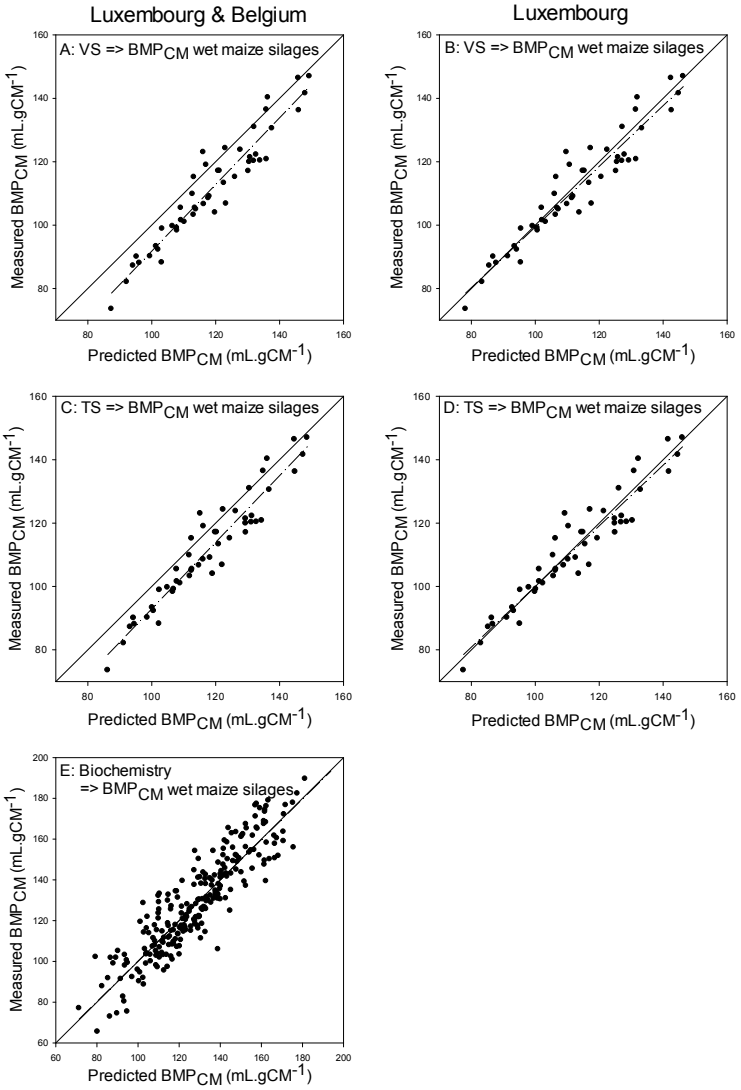

Figure 5.2. Measured and predicted biochemical methane potentials on a crude matter basis (BMP_{CM}) of wet maize silages with the volatile solids (VS, A and B) content or the total solids (TS, C and D) content or the biochemical composition (E). Calibration set of the model included samples cropped in Luxembourg and Belgium (A, C and E) or in Luxembourg only (B and D). Only results from the validation set are presented, except for the biochemistry model (E) where only a cross-validation was feasible and is presented. Solid line is the x = y line. Dashed line is the linear regression.

Table 5.3. Spearman's correlation coefficients between biochemical parameters and biochemical methane potentials (BMP) of wet maize silages.

Biochemical parameter	BMP_{CM} ($mL.gCM^{-1}$)	BMP_{VS} ($mL.gVS^{-1}$)
Volatile solids (%CM)	0.90*	-0.33*
Ash (%CM)	0.38*	-0.01
Cellulose (%TS)	-0.67*	0.12
Hemicellulose (%TS)	-0.38*	-0.02
Acid detergent lignin (%TS)	-0.47*	0.07
Crude proteins (%TS)	-0.35*	0.39*
Elemental C (%TS)	0.02	0.08
Fat (%TS)	0.17*	0.16*
Starch (%TS)	0.74*	-0.11

The BMP is expressed on a crude matter basis (BMP_{CM}) or on a volatile solids basis (BMP_{VS}). The statistics included 247 samples. Significant correlations are marked with a star ($p < 0.05$). TS: total solids.

The prediction of the BMP_{CM} of wet maize silages using the biochemistry model had a RPDC of 2.4 (Table 5.2). This calibration result was very close to the RPDC of 2.3 of VS model and TS model (Table 5.2). The detailed biochemical composition did not improve meaningfully the prediction of the BMP_{CM} of wet maize silages as compared to VS model and TS model.

The VIP scores of the biochemistry model (Figure 5.3) shows that the VS content is the most important parameter to predict the BMP_{CM}. Similarly to correlation coefficients (Table 5.3), the starch and cellulose contents were the following predictors with the highest VIP scores. Considering the low input of determining the individual biochemical fractions to predict the BMP on one hand and the time and resources required to measure these fractions of maize silages on the other hand, the detailed biochemical composition was not worth for the prediction of the BMP_{CM} of wet maize silages. The best method for a fast and accurate prediction of the BMP_{CM} of wet maize silages from biochemical analysis was to measure the TS content of the crude wet sample.

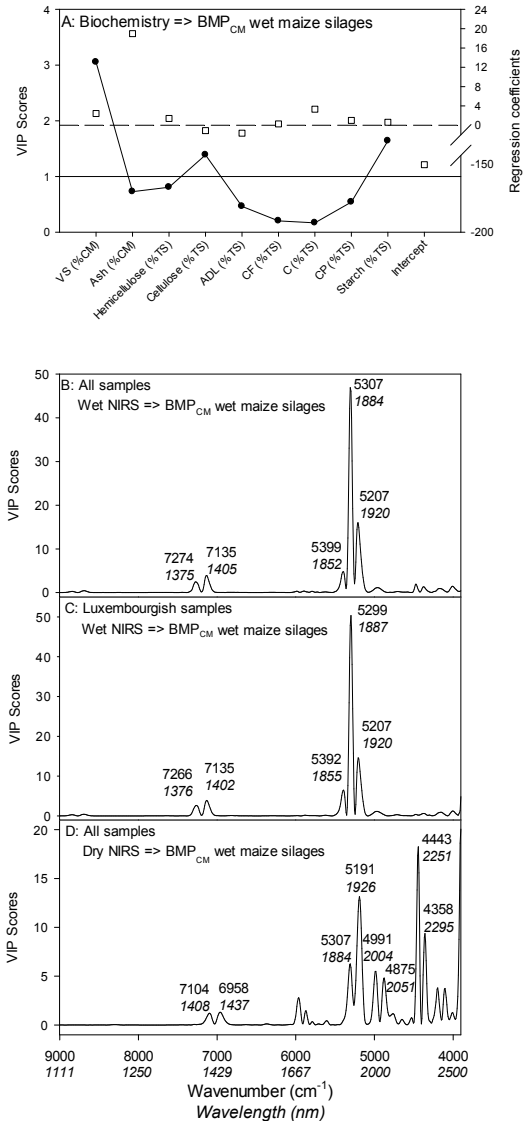

Figure 5.3. Variable importance in projection (VIP) scores of models that predict the biochemical methane potential on a crude matter basis (BMP_{CM}) of wet maize silages with the biochemical composition (A) or near infrared spectroscopy (NIRS). Wet NIRS (B and C) is NIRS measured on wet maize silages and dry NIRS (D) is NIRS measured on dry maize silage powders. In Figure A, VIP scores are presented as linked full circles and regression coefficients are presented as open squares. ADL: acid detergent lignin, CP: crude protein, C: elemental carbon, VS: volatile solids, CM: crude matter.

3.2. Influence of geographical origin of the sample

High biases of 7.2 and 6.1 mL.gCM^{-1} were observed when predicting the BMP_{CM} of the validation set from VS model and TS model respectively (Table 5.2 and Figure 5.2). The validation set was characterized by maize silages cropped only in Luxembourg, whereas maize silages of the calibration set were cropped in Luxembourg and in Belgium. For both calibration and validation sets, the BMP_{CM} and BMP_{VS} of the samples cropped in Luxembourg tended to be lower than the BMP_{CM} and BMP_{VS} of the samples cropped in Belgium (Figure 5.1). Prediction models associated to the cropping environment were thus investigated.

TS and VS models were calculated using only maize samples harvested in Luxembourg in the calibration set (Table 5.2, Figure 5.2). The biases were decreased with such an approach (biases of 1.1 and 0.5 mL.gCM^{-1} for VS model and TS model, respectively), as compared to models calibrated with maize samples from Luxembourg and Belgium. The CP and ash contents were shown to discriminate maize samples from Luxembourg and from Belgium and the maize maturity at the harvest could be different in both countries (Chapter 4, Figure 4.1). However, the CP and ash contents were not meaningfully affecting the prediction of the BMP_{CM} of wet maize silages, as described in Table 5.2 and Figure 5.3. The bias reduction could be explained by the use of samples harvested in similar fields at similar maturity for both calibration set and validation set.

3.3. Prediction of BMP from NIRS

Results of models based on NIRS and predicting the BMP of wet maize silages are presented in Table 5.4 and Figure 5.4. Prediction models using spectra measured on wet maize silages or on dry maize silage powders are identified below as wet NIRS or dry NIRS models.

Prediction of the BMP_{CM} of wet maize silages harvested in Luxembourg and in Belgium with a wet NIRS model showed a low SEP of 6.9 mL.gCM^{-1} (Table 5.4), but higher than the SEP of VS or TS-models (Table 5.2). However, NIRS is faster (few minutes) than measuring the VS or TS content of wet maize silages with the reference method (at least 24h). The BMP_{CM} of wet maize silages can be quickly and well predicted with NIR spectra of wet maize silages.

Table 5.4. Prediction models of the biochemical methane potential (BMP) of wet maize silages with near infrared spectroscopy (NIRS) or the biochemical compisiton.

Calibration and cross-validation						
Substrate	Wet maize silages					
Parameter	BMP_{CM}			BMP_{VS}		
Unit	$mL.gCM^{-1}$			$mL.gVS^{-1}$		
Predictor	Wet NIRS	Wet NIRS	Dry NIRS	Wet NIRS	Dry NIRS	Biochemistry
Country	All	Luxembourg	All	All	All	All
N	361	121	348	360	347	247
Outliers	1	0	1	1	1	0
Minimum	39	39	39	314	330	330
Mean	126	131	126	418	419	411
Maximum	201	201	201	557	557	534
SD	24.8	29	25	41	41	39.5
Reg. method	PLS	PLS	PLS	PLS	PLS	PLS
Range (cm^{-1})	9002-3903	9002-3903	9002-3903	9002-3903	9002-3903	-
Step (cm^{-1})	8	8	8	8	8	-
Predictor preprocess	2D(3;19); MC	2D(3;19); MC	SNV; Det; 2D(3;19);MC	2D(3;19); MC	SNV; Det; 2D(3;19);MC	AS
Cross-validation	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	-
LV	5	5	3	4	5	2
RMSEC	9.5	8.6	14.5	33.0	32.8	35.0
R^2C	0.85	0.91	0.65	0.35	0.35	0.21
RPDC	2.6	3.4	1.7	1.2	1.2	1.1
RMSECV	10.0	10.7	14.7	34.1	34.4	36.1
R^2CV	0.83	0.87	0.63	0.31	0.29	0.17
RPDCV	2.5	2.8	1.7	1.2	1.2	1.1
SEL prediction	3.2	5.5	2.2	5.9	6.5	-
Validation						
N	49	49	49	49	49	-
Outliers	0	0	0	0	0	-
Min	74	74	74	362	362	-
Mean	112	112	112	397	397	-
Max	147	147	147	449	449	-
SD	17	17	17	20	20	-
RMSEP	12.2	8.2	11.0	36.8	26.2	-
R^2P	0.84	0.84	0.59	0.15	0.06	-
RPDP	2.5	2.5	1.6	1.1	1.0	-
Bias	10.0	4.3	1.5	30.7	15.8	-
SEP	6.9	6.8	10.9	18.5	19.4	-
Intercept	-20.4	5.9	-2.3	209	254	-
Slope	1.09	0.91	1.01	0.44	0.34	-
Avg. T^2	2.6	6.5	4.5	2.0	5.8	-
SEL prediction	2.4	4.3	2.3	5.2	7.1	-

Wet NIRS is NIRS measured on wet maize silages and dry NIRS is NIRS measured on dry maize silage powders. Biochemistry includes VS, ash, cellulose, hemicellulose, acid detergent lignin, crude proteins, elemental C, fat and starch. 2D: 2nd derivative, Avg T^2: average Hotelling's T^2, Det: detrend, CM: crude matter, LV: latent variables, MC: mean-center, N: sample number, PLS: partial least squares, R^2C/CV/V: coefficient of determination of calibration/cross-validation/validation, RMSEC/CV/V: root mean square error of calibration/cross-validation/validation, RPDC/CV/P: ratio of standard error of prediction to sample standard deviation for calibration/cross-validation/prediction, SEL: standard error of laboratory, SEP: standard error of prediction, SNV: standard normal variate, VS: volatile solids.

A high bias of 10.0 mL.gCM^{-1} characterized the wet NIRS model using samples harvested in Luxembourg and in Belgium (Table 5.4, Figure 5.4). The calibration step was thus carried out with samples harvested only in Luxembourg, similarly to samples included in the validation set. With such calibration samples, the bias decreased to 4.3 mL.gCM^{-1} (Table 5.4). The BMP prediction of samples harvested in Luxembourg with a model calibrated with samples cropped only in Luxembourg improved the prediction accuracy. Indeed, a difference in term of biochemical composition was characterized in a previous chapter for maize silages harvested in Luxembourg or in Belgium (Chapter 3). The maturity at the harvest or the pedoclimatic conditions were considered to explain the difference between samples from the two countries.

The VIP scores of the wet NIRS models predicting BMP_{CM} are shown in Figure 5.3. The important spectral data were around 5307-5299 cm^{-1} and correspond to the absorbance of water molecules in the near infrared. They are similar to the VIP scores of the models that predict the VS and the moisture contents (Chapter 4, Figure 4.4). With near infrared spectra, the selected models predict the BMP_{CM} of wet maize silages thanks to the VS content of the biomass, which is totally correlated (r = -1.00) to the moisture content (Chapter 3, Table 5.2).

NIRS is a fast method to predict the BMP_{CM} of wet maize silages. In complement to laboratory analysis described in the present paper, NIRS can be implemented before the harvest in the field. The BMP_{CM} was predicted *in situ* from hyperspectral imaging (range: 450-2500 nm) taken during an overflight of fields (Udelhoven et al., 2013). Such an approach was found to be less accurate to predict the BMP_{CM} than spectroscopic measurements in the laboratory, but promising if performed with proper conditions.

NIRS was also used on-line in biogas plant to predict the biogas production of wet maize silages. Jacobi et al. (2012) measured a RMSECV of 11.1 L.kg^{-1} for biogas prediction, which is similar to the results presented here, considering that biogas is composed of around 50% of methane. A high correlation between VS and biogas production was also found. Spectroscopic measurements appear thus as promising analytical technics for agricultural biomethanation, from the field to the anaerobic digester.

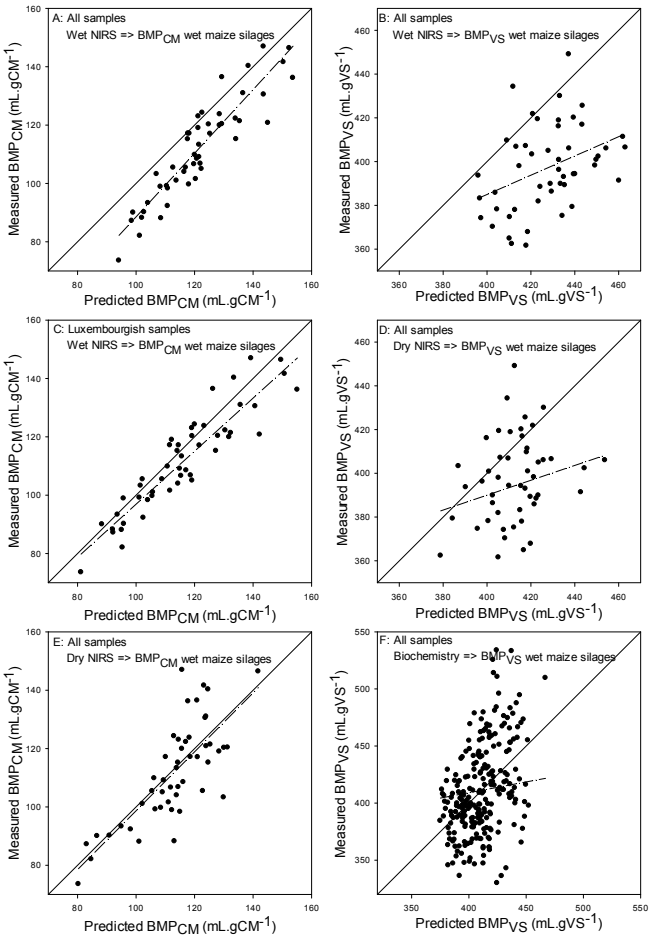

Figure 5.4. Measured and predicted biochemical methane potentials on a crude matter basis (BMP_{CM} ; A, C and E) or on a volatile solids content basis (BMP_{VS}; B, D and F) of wet maize silages with near infrared spectroscopy (NIRS) or the biochemical composition (biochemistry). Wet NIRS (A, B and C) was measured on wet maize silages and dry NIRS (D and E) was measured on dry maize silage powders. Biochemistry (F) includes volatile solids, ash, hemicellulose, cellulose, acid detergent lignin, crude proteins, elemental C, fat and starch contents. Calibration sets of the model included samples cropped in Luxembourg and Belgium (A, B, D, E and F) or only in Luxembourg (C). Results of the validation set are presented, except for the biochemistry model where only a cross-validation was feasible and is represented. Solid line is the x = y line. Dashed line is the linear regression.

The prediction of the BMP_{CM} with dry NIRS model is presented in Table 5.4 and Figure 5.4. The SEP of dry NIRS model was 10.9 $mL.gCM^{-1}$. Surprisingly, dry NIRS model was able to predict the BMP_{CM} of wet maize silages with an accuracy lower than wet NIRS model (Table 5.4).

The VIP scores of dry NIRS model predicting the BMP_{CM} of wet maize silages are presented in Figure 5.3. These VIP scores are similar to the VIP scores of dry NIRS model that predict the VS content of wet maize silage (Chapter 4, Figure 4.4). The prediction of the VS content of wet maize silages with dry NIRS model was explained by the correlation between the VS content and the cellulose content of maize silages (Chapter 4). The prediction of the BMP_{CM} of wet maize silage with dry NIRS is thus probably due to the good prediction of a biochemical fraction such as cellulose, which is correlated to the VS content of wet maize silages, itself correlated to the BMP_{CM} of wet maize silages.

The prediction of the BMP_{VS} of wet maize silages with wet NIRS, dry NIRS or biochemistry model is presented in Table 5.4 and Figure 5.4. Regardless the predictors (NIRS or biochemistry), the RPDCV and RPDP were lower than 1.2. The prediction of the BMP_{VS} of wet maize silages was very inaccurate in all cases. Such a poor prediction could be due to accuracy of the measurement method relative to the variability observed for the BMP_{VS}. The R^2_{max} of 0.72 for the prediction of BMP_{VS} was lower than the R^2_{max} for the prediction of BMP_{CM} (Table 5.1). Such poor result is due to a high error of measurement (SEL) as compared to the measured BMP_{VS} range (SD). The SEL/SD ratio for BMP_{VS} doubled as compared to the SEL/SD ratio for BMP_{CM} (53.4 and 21.2 respectively). The BMP_{VS} of wet maize silages cannot be precisely predicted because the repeatability of the BMP batch assay method is too low as compared to the variability of the measured BMP_{VS}. The repeatability of the BMP method must be improved to decrease the SEL and hypothetically to be able to predict the BMP_{VS} of wet maize silages. The heterogeneity of the maize silage samples added in the batch digesters can be responsible for such a low repeatability of BMP_{VS} measurement. Indeed, the particle size of wet maize silages is around 1 cm. A grounding step of the wet biomass could increase the homogeniety and then the repeatability of the BMP assay.

The prediction of BMP_{VS} of dry meadow grasses was already assessed and showed a RPDCV of 1.75 (Raju et al., 2011). Such higher results can be explained by the

higher homogeneity of the product, as compared to maize silages. However, the accuracy remained poor with a RMSECV of 37.4 mL.gVS^{-1}.

4. Conclusion

For maize varieties and harvest conditions investigated in the present study, the BMP_{CM} is mainly influenced by the total solids content of the substrate. The individual biochemical fractions do not have a major impact on the BMP_{CM}. NIRS and TS content measured on crude, wet matter allow to quickly predict the BMP_{CM} of wet maize silages with good accuracy (SEP of 5.2 and 6.9 mL.gCM^{-1}, respectively), similar to the BMP_{CM} repeatability (SEL of 5.3 mL.gCM^{-1}). For the tested maizes, the prediction of the BMP_{CM} of a sample is more accurate with a model that is calibrated with silage samples cropped in a similar environment. The prediction of BMP_{VS} of wet maize silage is not accurate regardless the predictor. This is due to the limited repeatability of the BMP method within the relatively narrow BMP_{VS} range observed for crude maize silages. Improvement of the BMP repeatability could increase the accuracy of the prediction.

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Highlights of Chapter 5

- The composition of the VS content of wet maize silages do not have a major impact on their BMP_{CM}.
- Models based on wet NIRS and TS content can predict the BMP_{CM} of wet maize silages.
- The cropping location of the maize silages affects the accuracy of the prediction of the BMP_{CM}.
- The prediction of the BMP_{VS} of wet maize silages is not accurate, regardless the predictor.

Chapter 6

Influence of sample preprocessing on the biochemical methane potential of maize silages and its prediction

The BMP of maize silages was measured using crude wet samples as substrates. The VS content was shown to influence the BMP_{CM} of wet maize silages (Chapter 3). The biochemical composition of maize silages was measured using dry maize silage powders. No significant influence of the biochemical composition was found on the BMP_{CM} of wet maize silages. However, BMP batch assays are often performed on the biomass processed as a dry powder, while the use of crude wet silages was preferred in the present work to reach practical purpose, as silages are used as is in biogas reactors. **What is the influence of drying and grinding wet maize silages on the measured BMP and its prediction with biochemical or spectral data?**

In the present chapter (Chapter 6), the BMP of maize silages is assessed using dry maize silage powders as substrates. It is compared to the BMP of the corresponding crude maize silages. Prediction of the BMP of dry maize silage powders is also investigated with models using the biochemical composition or NIRS, with special attention on the important spectral regions.

Abstract

The biochemical methane potential (BMP) assay is used to assess the production of biomethane of anaerobic digester feedstocks. For energy crops, the assay is commonly carried out on samples preprocessed as dried and ground matter.

Both crude wet maize silages and their respective dry maize silage powders were analysed in BMP batch assays in order to investigate the influence of drying and grinding wet maize silages. The BMP on a volatile solids (VS) basis was lower for dry maize silage powders than for wet maize silages. The loss of volatile compounds such as organic acids or alcohols during the drying of wet silages and the underestimation of the VS content of wet maize silages could explained the BMP difference between the two substrates.

The BMP measurement requires a time-consuming batch assay (50-60 days). Biochemical composition and near infrared spectroscopy (NIRS) were tested as predictors to estimate quickly the BMP of dry maize silage powders. The model based on the biochemical composition of maize silages successfully predicted the BMP_{CM} of dry maize silages with a RMSECV of 5 $mL.gCM^{-1}$. Starch and fibre contents affected positively and negatively, respectively, the BMP of dry maize silages powders. The model using NIR spectra of dry maize silage powders showed a RMSECV of 7 $mL.gCM^{-1}$. NIRS is less accurate but is a faster and easier way to estimate the BMP than using the biochemical composition. The prediction of the BMP_{VS} was less accurate than for the BMP_{CM}, regardless the predictor.

Keywords: bioenergy, biomass, energy crops, biochemical methane potential, BMP, near infrared spectroscopy, NIRS, biogas, biomethane, maize.

1. Introduction

Biomethanation is considered as an environmentally friendly way to produce energy through the anaerobic conversion of organic materials (European Commission, 2011). The anaerobic digestion consists in the degradation without oxygen of organic materials and their conversion into a biogas that contains methane. This biomethane is then valorised in heat, electricity or fuel (Deublein & Steinhauser, 2008).

A wide variety of organic materials has been used for decades as substrates to feed anaerobic digesters and produce biomethane (Shiralipour & Smith, 1984). The amount of biomethane produced per unit of matter is defined as the biochemical methane potential (BMP). The BMP differs from one sample to another (Raposo et al., 2012).

A laboratory batch digestion assay is the most common method to measure and assess the BMP. The principle consists in incubating a sample with an anaerobic inoculum in a reactor without oxygen and measure the methane gas production over time (up to 50-60 days). Behind such an apparent simplicity, various factors affect the BMP assay (Raposo et al., 2011; Raposo et al., 2012).

Among these factors, the inoculum to substrate ratio (ISR) was the main factor evaluated (Chen & Hashimoto, 1996; Dechrugsa et al., 2013; González-Fernández & García-Encina, 2009; Hashimoto, 1989; Raposo et al., 2006). On the other hand, the influence of sample preprocessing was less often assessed (Ohl & Hartung, 2010; Mshandete et al., 2006).

The BMP measurement on a volatile solid basis (BMP_{VS}) of wet maize silages had a low repeatability as compared to its variability (Mayer et al., 2014). The particle heterogeneity of wet maize samples resulting in variability in the subsampling could be responsible for such a low repeatability. Consequently, the calibration of an accurate model that predicts the BMP_{VS} of wet maize silages with their biochemical composition or data from near infrared spectroscopy (NIRS) was limited (Chapter 4).

The influence of drying and grinding on the BMP measurement of maize silages was assessed in the first part of the present chapter. In a second part, prediction of the

BMP of dry maize silage powders was investigated with models using the biochemical composition or NIRS as predictors.

2. Materials and methods

2.1. Maize production

Maize was produced as described by Mayer et al. (2014a, 2014b). In brief, various maize varieties (FAO index between 240 and 340) were cropped in various fields in Luxembourg and in Belgium between 2007 and 2011. The harvest was realized at different maturities to enhance the biomass composition variability. The aerial part of maize was harvested with a mechanical harvester (Haldrup, Inotech Engineering GmBH, Germany) and chopped to a particle size of around 1 cm. After harvest, the wet chopped biomass was packed in plastic bags and sealed under vacuum to allow silaging. Fermentation gas produced during silaging was removed by opening the bag 2-3 days after first packing and resealing it under vacuum. After stabilisation, wet maize silages were stored at room temperature until further analysis.

2.2. Sample preprocessing

A subsample of each wet maize silage was dried for 48h in an oven at 70°C. The dry matter was subsequently ground in a centrifugal mill (Cyclotec, Foss) through a 1-mm mesh. Dry maize silage powders were stored in closed plastic bags until laboratory analysis.

2.3. Biochemical measurements

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

For dry maize silage powders, a mean value of 9.1%CM of moisture, obtained from previous experiments (data not shown), was used to measure the TS content. The VS content of dry maize silage powders was quantified from the ash content of wet maize silages.

The methods to measure the biochemical composition of maize silages were precisely described in Chapter 4. Cellulose, hemicellulose, acid detergent lignin (ADL), fat, elemental N and C were measured in dry maize silage powders. Cellulose, hemicellulose and ADL were measured after digestions in an automatic analyser (Ankom 2000, Ankom Technology) or in beakers (for ADL only) using methods adapted from Van Soest (Van Soest et al., 1991) and modified for starch removal (Mertens, 2002). The fat content was extracted with hexane in a Soxhlet extractor (Buchi) and gravimetrically quantified after a solvent evaporation (AOAC International, 2006). Elemental C and N were measured with a TruSpec elemental analyser (LECO) and expressed as a percentage of the TS content. The crude proteins (CP) content was calculated by multiplying the elemental N content by 6.25 (Jones, 1931). Starch was predicted with a validated model using near infrared spectra of dry maize silage powders in the *Centre walloon de Recherches agronomiques* (Gembloux, Belgium).

2.4. NIRS measurements

NIR spectra of both wet maize silages (wet NIRS) and dry maize silage powders (dry NIRS) were measured before carrying out the BMP batch assays, as described precisely in Chapter 4. The residual humidity content of dry maize silage powders was note removed. In brief, a sampling cup was filled with the wet maize silage or the dry and ground maize silage to measure the NIR reflectance spectrum of the sample with a Bruker MPA spectrometer (Bruker Optik GmbH) operated with OPUS 6.5. This procedure was realized three times per sample to obtain triplicate measurements. Absorbance spectra were collected in the 3594-9989 cm^{-1} range with a resolution of 16 cm^{-1}. The measured NIR spectrum of a sample was the result of the average of 32 spectra automatically collected during the rotation of the sample cup.

2.5. BMP measurements

The method to measure the BMP was precisely described by Mayer et al. (2014b), following the recommendations of the VDI 4630 standard (Verein Deutscher Ingenieure, 2006). In brief, batch assays were carried out in 2L bottles. The digesters

were filled with an inoculum sampled in an anaerobic bioreactor of a wastewater treatment plant (SIVEC, Schifflange, Luxembourg) and with the sample to analyse (crude wet maize silage or dry maize silage powder). The produced biogas was collected in gas bags through cooled tubes to condense water vapour. The collected biogas was regularly and automatically analysed for its volume and composition, with a TG05 wet drum-type gasmeter (Ritter, Germany) and specific infrared sensors (Dynament, UK) respectively. Gas volumes were normalised (273 K, 1013 hPa) according to the temperature and pressure conditions. The endogenous gas production of the inoculum was subtracted for each gas measurement. BMP measurements were carried out in triplicates for all samples. The inoculum activity was assessed with BMP assays of microcrystalline cellulose run simultaneously.

The BMP was calculated after 56 days at the end of the batch assays as the cumulated biomethane production. The BMP were expressed relatively to the crude matter of wet maize silages or dry maize silage powders added in the digester (BMP_{CM}) and then expressed per unit of volatile solids (BMP_{VS}).

2.6. Data treatment and chemometrics

The number of samples, the mean, the standard deviation (SD) and the standard error of laboratory (SEL) were calculated with Excel 2010 (Microsoft Corp.).

Since both BMP measurements, using wet maize silages or dry maize silage powders as substrates, have measurement errors, the Deming regression (Deming, 1943) was used instead of classic linear regression to compare BMP of the two types of substrate. Bland-Altman graph (Altman & Bland, 1983) helped to determine if two measurement methods gave the same results. Boxplots, Bland-Altman graph, Deming regression, linear regressions and confidence intervals were calculated with SigmaPlot 12.5 (Systat Software).

Statistical analyses were carried out with SPSS, version 19 (SPSS Inc.). Normal distribution of a population and homogeneity of variances were assessed with the Shapiro-Wilk test and the Levene statistic respectively before comparing means. The mean comparison between two groups was realized with a Student test or a Kruskal-Wallis test if normality hypothesis was violated. Tukey or T3-Dunnet post-hoc tests were used in case of equal or unequal variances, respectively. An α-risk of 0.05 was used as the significant probability level for all statistical tests.

Model calculations were carried out with Matlab, version R2012a (MathWorks) and PLS_Toolbox, version 7.3.1 (Eigenvector Research Inc.).

Before any model calculation, the maximum coefficient of determination of any calibration (R^2_{max}) was calculated according to Dardenne (2010) using:

$$R^2_{max} = \frac{SD^2 - SEL^2}{SD^2}$$

For the modelling procedure, the triplicate NIR spectra of a sample were averaged to obtain one single spectrum per sample. Triplicate and average spectra were plotted to check outliers before being used in the model calculation as predictors. The regression method used was the partial least squares (PLS) for all models.

Preprocessing methods included mean centering (MC), autoscaling (AS), standard normal variate (SNV) or detrend (Det). Derivatives were also assessed and abbreviated as xD(y;z), with x the derivative order, y the polynomial order and z the filter width. The excluded spectral data were used in the derivative calculations, but not in the subsequent model.

A cross-validation was used during the calibration procedure to find the optimal number of latent variables (LV) for multivariate models. The cross-validation consisted in the random split of the calibration set in 10 segments, with the same number of samples in each segment. The calibration model was then calculated with all the samples from 9 segments. Using this calibration model, the value of the parameter was predicted for each sample of the 10th segment. This procedure was repeated 10 times to calculate the statistics of the model.

The root mean square error (RMSE), the coefficient of determination (R^2) and the ratio of standard error of prediction to sample standard deviation (RPD) was calculated for the calibration (RMSEC, R^2C, RPDC) and cross-validation (RMSECV, R^2CV, RPDCV).

The RMSE was calculated according to:

$$RMSE = \sqrt{\frac{\sum(y_m - y_p)^2}{N}}$$

with y_m the measured values, y_p the predicted values and N the number of samples in the dataset.

The R^2 was calculated according to:

$$R^2 = \left(\frac{\sum(\widehat{y_m} - \overline{y_m})^2}{\sum(y_m - \overline{y_m})^2}\right)$$

with y_m the measured values, $\widehat{y_m}$ the estimated y_m values given by the regression line and $\overline{y_m}$ the mean y_m value.

The RPD, which is a more discriminant value than the R^2 value, was calculated according to:

$$RPD = \frac{1}{\sqrt{1 - R^2}}$$

The intercept and the slope were calculated for the linear regression between the predicted value (x-axis) and the measured value (y-axis) for the cross-validation data. The best model was selected and presented in the present paper according to the lowest RMSECV, the lowest number of LV and the highest R^2CV and RPDCV.

The variable importance in projection (VIP) scores give a summary of the importance of a predictor for both predictors and predicted parameter. A variable with a VIP score close to or greater than 1 can be considered as important in a given model (Wold et al., 2001). The VIP score for the *j*-th variable was calculated according to:

$$VIP_j = \sqrt{p \frac{\sum_{k=1}^{h}\left(b_k^2 t_k^t t_k \left(\frac{w_{jk}}{\|w_k\|}\right)^2\right)}{\sum_{k=1}^{h} b_k^2 t_k^t t_k}}$$

with p the number of predictors, h the number of LV, k the k-th LV, b the regression coefficient of the score matrix, t the column vector of the score matrix, w the column vector of the weight matrix (Chong & Jun, 2005).

The SEL of prediction of each parameter for the calibration set and the validation set was calculated by applying the model to spectra replicates in order to obtain the predicted values.

3. Results and discussion

3.1. Influence of drying and grinding on the BMP

The BMP_{VS} of wet maize silages and their respective dry powders (N = 48 for each) were analysed in 4 series of batch assays. The corresponding wet and dry samples were analysed in the same batch series to avoid the effect of different inocula. The BMP_{VS} of both forms of substrate are presented in Figure 6.1. Wet maize silages showed higher BMP_{VS} on average than dry maize silage powders. The difference was 19 $mL.gVS^{-1}$, corresponding to 5% of the average BMP_{VS} of wet maize silages. According to the Deming regression slope (1.08) and the Bland-Altman graph, this difference was similar on average along the measured range of BMP_{VS}. The BMP_{VS} was calculated from the measured BMP_{CM} and the VS content of wet maize silages. However, the VS content is underestimated because volatile organic compounds (VOC), mostly organic acids and alcohols, were lost during the drying step of wet maize silages to measure the VS content. Nevertheless this volatile organic matter was present and easily converted in biomethane when digesting wet maize silages. The calculated BMP_{VS} of wet maize silages is then overestimated through an underestimation of the substrate organic matter, characterized by the VS content.

A drying step was also required to produce the dry maize silage powders. VOC are expected to be lost in such a substrate. Indeed, Weißbach and Strubelt (2008) measured in wet maize silages (mean TS of 33.7 $\%CM^{-1}$) a mean concentration of 3.2 $\%CM^{-1}$ of acids and alcohols, mainly acetic acid (0.998 $\%CM^{-1}$), lactic acid (1.520 $\%CM^{-1}$) and ethanol (0.580 $\%CM^{-1}$). According to theoretical COD of these compounds, such concentrations can produce around 14 $mL.gCM^{-1}$ of methane, which corresponds to 42 $mL.gVS^{-1}$ for a maize silage having a VS content of 33 $\%CM^{-1}$. VOC of wet maize silages that were lost in dry maize silage powders can explain the lower BMP_{VS} measured for dry maize silage powders, as compared to wet maize silages.

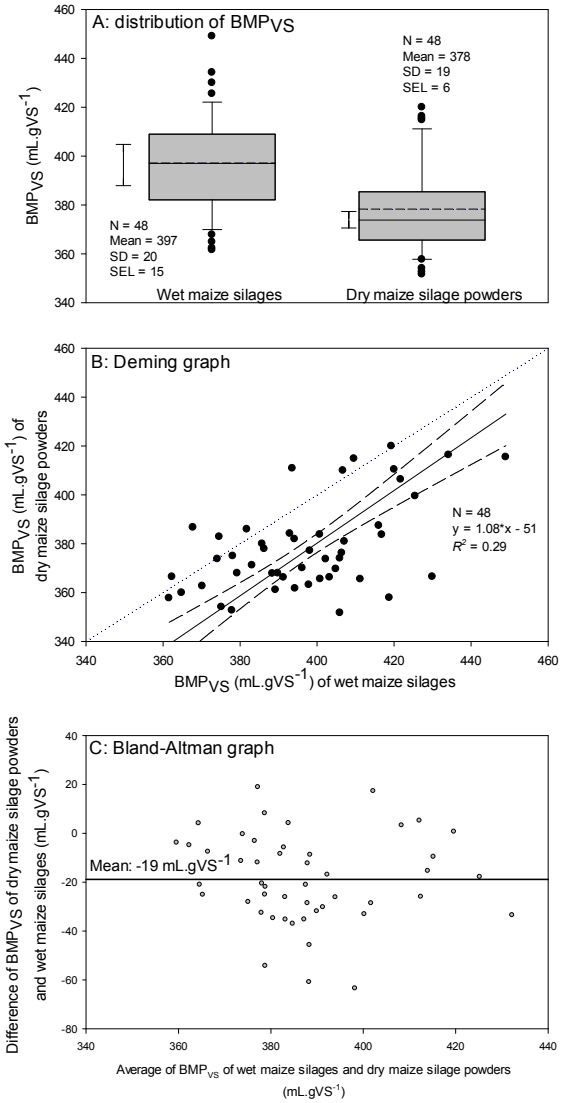

Figure 6.1. Distribution (A), Deming regression (B) and Bland-Altman graph (C) for the biochemical methane potentials on a volatile solids content basis (BMP_{VS}) measured on wet maize silages and on dry maize silage powders. In graph A , standard error of laboratory (SEL) is represented by a vertical bar on the left of the box plots. The mean is represented by dashed line in the box plots. In graph B, solid line represents the Deming regression. Dotted line is the y = x axis. Dashed lines represent the 95% confidence interval. SD: standard deviation, N: number of samples, R^2: coefficient of determination.

Figure 6.1 shows that the variability of the BMP_{VS} was similar for both forms of substrate (SD around 19-20 $mL.gVS^{-1}$) but the repeatability was better with dry maize silage powders (SEL of 6 $mL.gVS^{-1}$) than with wet maize silages (SEL of 15 $mL.gVS^{-1}$). The grinding step in centrifugal mill increases the homogeneity of the sample, as compared to the initial wet maize silage wherein maize grain or leave could still be identified. The higher homogeneity of the sample can be responsible for the lowest SEL observed for dry maize silage powders as compared for wet maize silages. The BMP should be measured on wet silage samples that are ground to digest all organic compounds and obtain homogeneous and representative subsamples.

In a similar study, Ohl and Hartung (2010) observed opposite results: the BMP_{VS} of dry maize silage powders were higher than those of wet maize silages, by a factor of 1.14 on average. The VS content was also not corrected for losses of VOC. They explained such results by a better homogenization of the sample and a greater availability of nutrients for bacteria in dry maize silage powders. However, they used different scientific instruments to measure the BMP of each form of substrate. Different results were found in the present study, using the same instrumental devices to carry out the BMP assays for two different maize preprocessings. The instrumentation used to measure the BMP can have an effect on the measurement.

3.2. Influence of the VS content of wet maize silages on BMP

Figure 6.2 shows the influence of the VS content of wet maize silages on the BMP_{VS} of dry maize silage powders. The anaerobic digestibility of dry maize silage powders was similar at different VS contents of the wet silages. On the contrary, the BMP_{VS} measured on wet maize silages (Figure 3.3) or on various plant species (Figure 7.4) were observed to decrease with increasing VS content (Mayer et al., 2014).

The BMP_{VS} of dry maize silage powders was well measured since most of the volatile organic compounds were lost during the drying step and were not included in the VS measurement of dry maize silage powders. This is not the case for the measurement of the BMP_{VS} of wet maize silages. Indeed, the wet biomass contains

volatile organic metabolites. It was shown in wet maize silages that the total amount of water soluble carbohydrates and organic acids decreases with maturity while the dry matter content increases (Filya, 2004). The VS content of wet maize silages also increases over cropping time and can be used as a plant maturity indicator (Schittenhelm, 2008 ; Gao et al., 2012; Mayer et al., 2014). Consequently, low VS contents are more underestimated than high VS contents, because of high contents of volatile compounds at early maturity stages. The overestimation of the BMP_{VS} of wet maize silages is thus higher at low VS content than at high VS content. For wet maize silages, the VS content should be corrected before calculating the BMP_{VS}. The decreasing trends observed in Figure 3.3 and in Figure 7.4 can be influenced by the misestimation of the VS content measurement and the BMP_{VS} calculation. The identification and quantification of the volatile compounds lost during the drying step should be investigated to correct the VS content of wet maize silages. Then, the measurement of BMP_{VS} of wet maize silages should be rectified according to the corrected VS measurements in order to highlight the influence of the maturity on the BMP of wet maize silages.

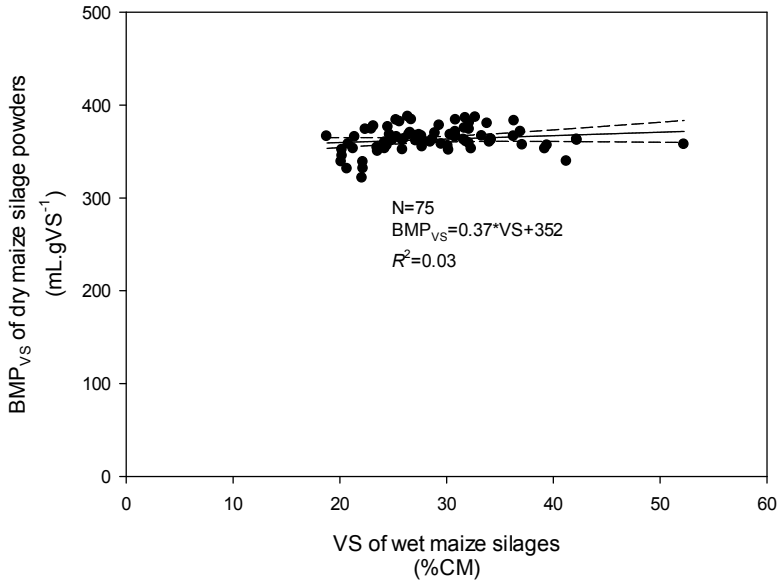

Figure 6.2. Influence of the volatile solids (VS) content of wet maize silages on the biochemical methane potential on a VS content basis (BMP_{VS}) of dry maize silage powders. N: number of samples, R^2: coefficient of determination.

3.3. Prediction of BMP

The prediction of the BMP of dry maize silage powders was assessed with models using the biochemical composition or NIRS as predictor. The BMP was measured on 75 dry maize silage powders, out of which 29 samples were analysed for their biochemical composition. Both measured BMP_{CM} and BMP_{VS} of dry maize silage powders are described in Table 6.1.

The R^2_{max} for BMP_{CM} of dry maize silage powders (R^2_{max} = 0.90, Table 6.1) was lower than the R^2_{max} for BMP_{CM} of wet maize silages (R^2_{max} = 0.96, Table 5.1). Despite a better SEL for dry maize silage powders (SEL = 4.8 $mL.gCM^{-1}$) than for wet maize silages (SEL = 5.3 $mL.gCM^{-1}$, Table 5.1), the BMP_{CM} range observed for dry maize silage powders (58 $mL.gCM^{-1}$) was lower than for wet maize silages (161 $mL.gCM^{-1}$). The narrow range measured for BMP_{CM} of dry maize silage powders was responsible for the lowest R^2_{max}, as compared to the R^2_{max} calculated for the BMP_{CM} of wet maize silages.

However, the R^2_{max} of BMP_{VS} was better for dry maize silage powders (R^2_{max} = 0.83) than for wet maize silages (R^2_{max} = 0.72, Table 5.1). In this case, the low SEL measured for dry maize silage powders allows higher R^2_{max} than for BMP_{VS} of wet maize silages. Models that predict the BMP_{VS} should be more accurate with dry maize silage powders than with wet maize silages.

Prediction of BMP from biochemical parameters

Selected models that predict BMP from biochemical parameters are presented in Table 6.1 and Figure 6.3. For both BMP_{CM} and BMP_{VS}, accurate prediction results (RMSECV of 5.2 and 7.2 $mL.g^{-1}$ respectively) were observed for models using biochemistry as predictor. The biochemical composition has an effect on the BMP of dry maize silage powders and thus, it can be used as a fast predictor of the BMP of maize silages.

The correlation between biochemical parameters and BMP are presented in Table 6.2. The BMP_{CM} of dry maize silage powder was anticorrelated with cellulose (r = -0.72), hemicellulose (r = -0.72) and ADL (r = -0.64), and correlated with starch (r = 0.80). Moreover, the VIP scores and regression coefficients of biochemistry models, presented in Figure 5.4, shows similar results.

Table 6.1. Prediction models of the biochemical methane potential (BMP) of dry maize silage powders with near infrared spectroscopy (NIRS) or the biochemical composition.

Calibration and cross-validation						
Substrate	Dry maize silage powder					
Parameter	BMP_{CM}			BMP_{VS}		
Unit	$mL.gCM^{-1}$			$mL.gVS^{-1}$		
Predictor	Wet NIRS	Dry NIRS	Biochemistry	Wet NIRS	Dry NIRS	Biochemistry
N	75		29	75		29
Outliers	0		0	0		0
Minimum	283		283	321		321
Mean	315		304	363		354
Maximum	341		320	387		371
SD	14.9		11.1	13.3		12.0
RSD (%)	4.7		3.6	3.7		3.4
SEL	4.8		3.7	5.5		4.3
RSEL (%)	1.5		1.2	1.5		1.2
SEL/SD (%)	32		33.5	41		36.0
R^2_{max}	0.90		0.89	0.83		0.87
Reg. method	PLS	PLS	PLS	PLS	PLS	PLS
Range (cm^{-1})	9002-3903	9002-3903	n.a.	9002-3903	9002-3903	n.a.
Step (cm^{-1})	8	8	n.a.	8	8	n.a.
Predictor Preprocess	2D(3;9); MC	SNV; Det; 2D(3;19); MC	AS	2D(3;9); MC	SNV; Det; 2D(3;19); MC	AS
Cross-validation	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)
LV	6	4	3	4	4	3
RMSEC	6.3	6.0	4.1	8.7	6.6	5.7
R^2C	0.82	0.83	0.88	0.57	0.75	0.77
RPDC	2.4	2.5	2.9	1.5	2.0	2.1
RMSECV	10.8	6.7	5.2	11.1	7.5	7.2
R^2CV	0.50	0.80	0.81	0.32	0.68	0.64
RPDCV	1.4	2.2	2.3	1.2	1.8	1.7
Slope	0.85	0.97	1.00	0.83	0.96	0.95
Intercept	46.4	8.5	-1.1	62.1	16.0	17.1
SEL prediction	9.0	1.4	-	4.7	1.1	-

Wet NIRS was measured on wet maize silages and dry NIRS was measured on dry maize silage powders. The biochemical composition (biochemistry) includes VS, ash, cellulose, hemicellulose, acid detergent lignin, crude proteins, elemental C, fat and starch. 2D: 2nd derivative, AS: autoscale, CM: crude matter , Det: detrend, LV: latent variables, MC: mean-center, N: sample number, PLS: partial least squares, R2C/CV: coefficient of determination of calibration/cross-validation, RMSEC/CV: root mean square error of calibration/cross-validation, Rdm: random, RPDC/CV: ratio of standard error of prediction to sample standard deviation for calibration/cross-validation, SD: standard deviation SEL: standard error of laboratory, SEP: standard error of prediction, SNV: standard normal variate, VS: volatile solids.

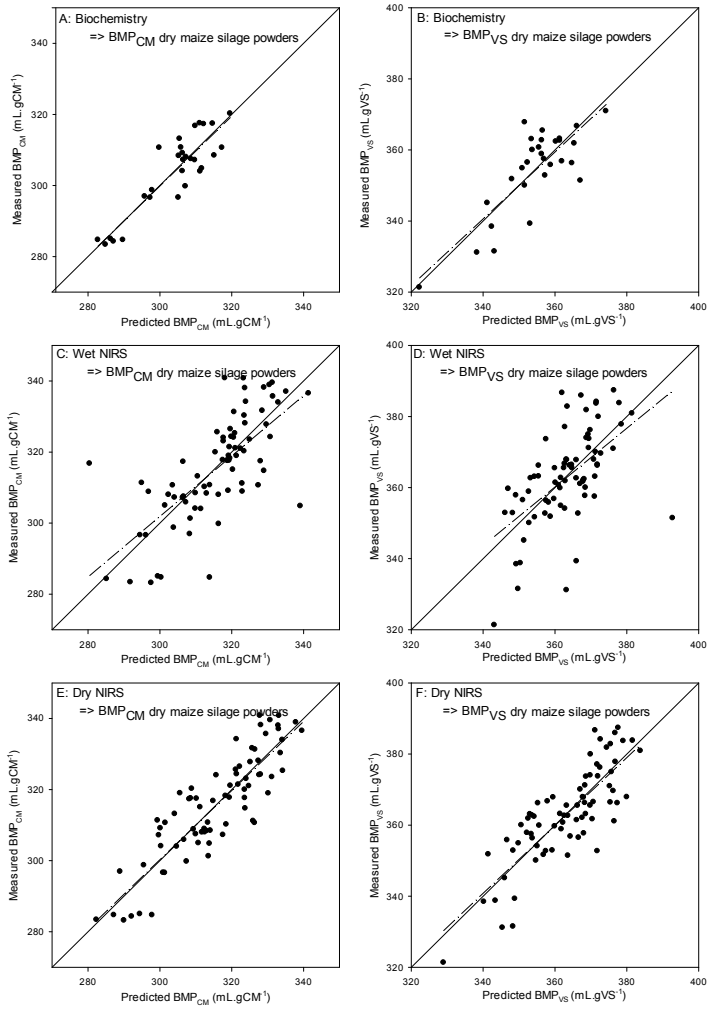

Figure 6.3. Measured and predicted biochemical methane potentials on a crude matter basis (BMP_{CM}; A, C and E) or on a volatile solids content basis (BMP_{VS}; B, D and F) of dry maize silage powders with near infrared spectroscopy (NIRS) or the biochemical composition (biochemistry). Biochemistry (A and B) includes ash, hemicellulose, cellulose, acid detergent lignin, crude proteins, elemental C, fat and starch.Wet NIRS (C and D) was measured on wet maize silages and dry NIRS (E and F) was measured on dry maize silage powders. Results from the cross-validation are presented. Solid line is the x = y line. Dashed line is the linear regression.

The BMP_{CM} prediction of dry maize silage powders was mainly due to the fibres (cellulose, hemicellulose and ADL), that have negative regression coefficients and starch showing a positive regression coefficient. Starch and fibres appear as the main parameters to measure to predict the BMP of dry maize silage powders.

The characteristics of the best maize silage for biomethane production can be highlighted with the regression coefficients of the biochemistry model. The regression coefficient for starch is positive whereas the regression coefficients were negative for the three fibre fractions. Starch increased the BMP, whereas fibres limited the anaerobic digestibility of dry maize silage powders. Starch increases with maturity of maize while the relative fibre content decreased (Godin et al., 2013b). From this observation, mature maize with high content of starch should be cropped to obtain high biomethane production in anaerobic digesters. However, the effect of the VOC content of wet maize silages was not assessed and the BMP_{VS} did not significantly increase with the VS content. No clear conclusions about the characteristic of the biochemical composition of the best maize for anaerobic digestion can be stated so far. Moreover, high BMP does not mean high biomethane yield per hectare since the biomethane yield per hectare was mainly influenced by the biomass yield per hectare (Mayer et al, 2014).

The influence of biochemical parameters on the BMP of maize has already been assessed with prediction models. Whereas Grieder et al. (2011) did not succeed to predict the BMP with chemical parameters, Rath et al., (2013) found that hemicellulose was the most significant parameter affecting positively the BMP_{VS} of non-ensiled dry maizes. Results presented here showed that hemicellulose had a negative influence on the BMP. The equation of Weissbach to predict the BMP also had a negative coefficient for crude fibers (Grieder et al., 2011). Some research work is still required to define which biochemical parameters are important for biomethane production of maize and what is the influence of the characteristics of the samples, such as the cropping environment, the maturity stage or the variety.

The prediction of BMP_{VS} of dry maize silage powders was poorer than the prediction of BMP_{CM}, as anticipated with the R^2_{max} values. The inaccuracy of the ash measurement (Table 4.1) or the use of a mean value for the residual moisture can have an effect on the calculation of the BMP_{VS} and can be responsible of such results.

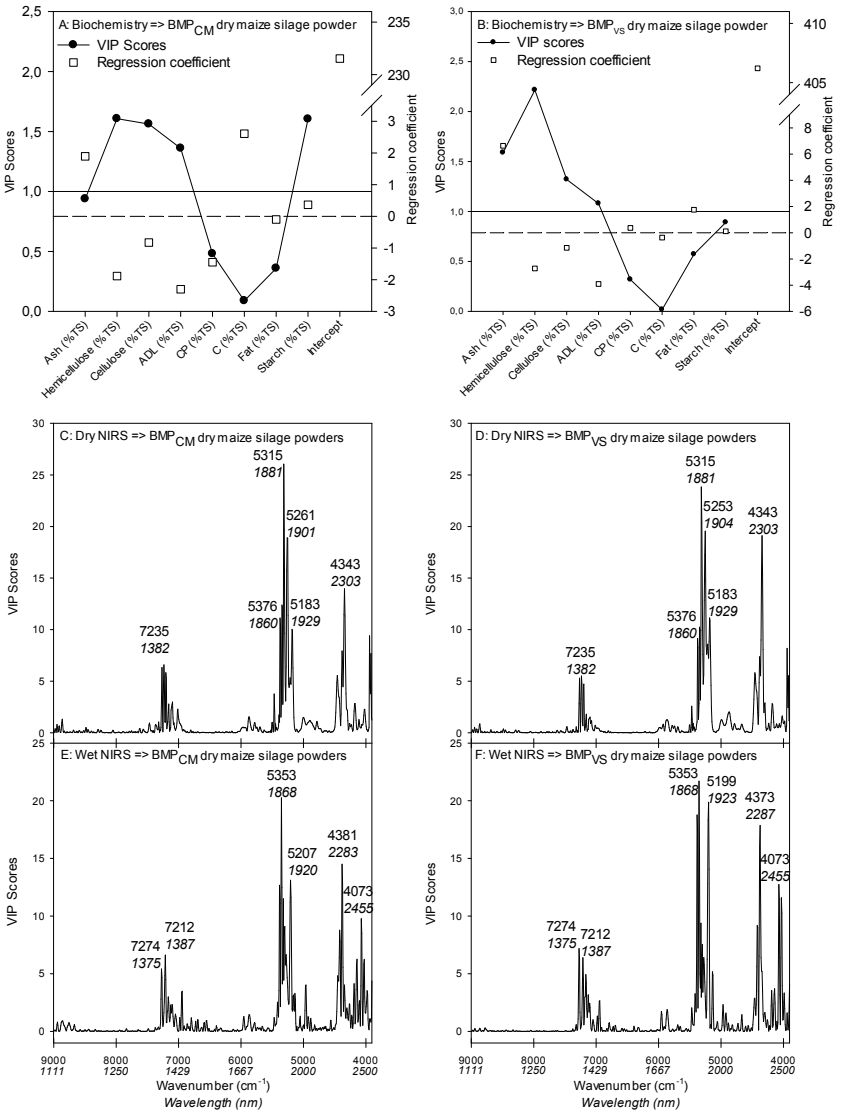

Figure 6.4. Variable importance in projection (VIP) scores of models that predict the biochemical methane potential (BMP) on a crude matter basis ($BMP_{CM;}$ A, C and E) or on a volatile solids content basis (BMP_{VS}; B, D and F) of dry maize silage powders with near infrared spectroscopy (NIRS) or the biochemical composition (biochemistry). Wet NIRS (E and F) was measured on wet maize silages and dry NIRS (C and D) was measured on dry maize silage powders. VS: volatile solids, ADL: acid detergent lignin, CM: crude matter, CP: crude protein, C: elemental carbon.

Table 6.2. Spearman's correlation coefficients between biochemical parameters and biochemical methane potentials (BMP) of dry maize silage powders.

Parameter	BMP_{CM} ($mL.gCM^{-1}$)	BMP_{VS} ($mL.gVS^{-1}$)
Ash (%TS)	0.28	0.21
Cellulose (%TS)	-0.72*	-0.53*
Hemicellulose (%TS)	-0.72*	-0.66*
Acid detergent lignin (%TS)	-0.64*	-0.45*
Crude proteins (%TS)	-0.45*	-0.16
Elemental C (%TS)	0.27	0.21
Fat (%TS)	0.40*	0.47*
Starch (%TS)	0.80*	0.56*

BMP are expressed on a crude matter basis (BMP_{CM}) or on a volatile solids content basis (BMP_{VS}). TS: total solids. The calculation included 29 samples. Significant correlations are marked with a star ($p < 0.05$).

Prediction of BMP from NIRS

The prediction of the BMP of dry maize silage powders with NIRS was assessed and is presented in Table 6.1 and Figure 6.3. Prediction of BMP_{CM} of dry maize silage powders with dry NIRS showed a low RMSECV of 6.7 $mL.gCM^{-1}$ and a high RPDCV of 2.2. Dry NIRS appears as an accurate predictor of the BMP_{CM} of dry maize silage powders, as compared to the SEL of the BMP measurement (4.8 $mL.gCM^{-1}$). Similar prediction results (RMSECV of 6.22 and R^2CV of 0.76) were reported for the BMP prediction of dry maize silage powder with a different BMP assay instrumentation (Grieder et al., 2011).

Wet NIRS (RPDCV of 1.4) was a poorer predictor of the BMP_{CM} of dry maize silage powders than dry NIRS. The presence of water and the heterogeneity of the material presented to the spectrometer interfere with the signal acquisition for wet maize silages, as already observed in Chapter 4. More spectral replicates could improve the prediction from wet NIRS models (Jacobi et al., 2011).

The BMP_{VS} prediction was less accurate according to the RPDCV of 1.8. Similar decrease of the accuracy of the prediction was observed between BMP_{CM} and

BMP_{VS} for the model that used the biochemical composition. It was explained by the inaccuracy of the ash measurement. Similarly for wet maize silages, the prediction of BMP_{VS} was less accurate than the prediction of BMP_{CM} (Chapter 4). It is then advised to predict the BMP_{CM} of the substrate and calculate the BMP_{VS} of the sample with its VS content in a second step.

The VIP scores of the models that used NIRS as predictor are presented in Figure 6.4. The VIP scores of wet NIRS and dry NIRS were similar regardless the predicted variable (BMP_{CM} or the BMP_{VS} of dry maize silage powders) for both NIRS models. They changed according to the preprocessing of the maize silages (wet NIRS or dry NIRS). No spectral information allowed discriminating the BMP_{CM} from the BMP_{VS} values, since they were very close.

Important VIP scores of models predicting the BMP of dry maize silage powders were in the range of 5376-5183 cm^{-1} and 4381-4343 cm^{-1}. Such ranges include water absorption around 5307 cm^{-1}. Cellulose and hemicellulose, which are important parameter for the BMP prediction, have a peak of VIP scores at 4366 cm^{-1} (Figure 4.7). The VIP scores of the prediction of BMP of dry maize silage powders with NIRS do not correspond exactly with the VIP scores of the model predicting the fibres, which are the important biochemical parameters for BMP prediction. Important spectral parameters that allowed the prediction of the BMP could not be well related to biochemical information.

4. Conclusion

The preprocessing of the sample analyzed in the BMP assay had an effect on the BMP measurement. Dry maize silage powders have lower BMP on a VS basis than wet maize silages. This result can be explained by the loss of volatile organic substances (ethanol, lactic acid, volatile fatty acids…) during the drying step to produce the dry maize silage powders and the overestimation of the BMP_{VS} of wet maize silages owing to the underestimation of the VS content. The repeatability of the BMP_{VS} measurement was better with dry maize silage powders than with wet maize crude silages. Biochemical composition and NIRS can be used to accurately predict the BMP of dry maize silage powders. Ground wet silages should be tested as substrates for the BMP measurement.

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Highlights of Chapter 6

- The BMP_{VS} of dry maize silage powders is lower than the BMP_{VS} of their corresponding crude wet maize silages.
- The VS content of wet maize silages does not have an effect on the BMP_{VS} of dry maize silage powders.
- Biochemical composition and NIRS can be used to accurately predict the BMP of dry maize silage powders.
- The repeatability of the BMP_{VS} measurement is better with dry maize silage powders than with wet maize crude silages.

Chapter 7

Assessment of energy crops alternative to maize for biogas production in the Greater Region

Adapted from:

Mayer F., Gerin P. A., Noo A., Lemaigre S., Stilmant D., Schmit T., Leclech N., Ruelle L., Gennen J., von Francken-Welz H., Foucart G., Flammang J., Weyland M. & Delfosse P. (2014). Assessment of energy crops alternative to maize for biogas production in the Greater Region. *Bioresource Technology*, 166, 358-367. doi: 10.1016/j.biortech.2014.05.054.

So far, the biomethane yield and the prediction of the BMP were investigated for maize silages specifically. However, maize is not the only energy crop that can be used in biomethanation. Other plant species are also cropped and harvested for biomethane production. **What is the influence of the plant species on the biomethane yield per hectare?**

In the present chapter (Chapter 7), other energy crops cropped in the Greater Region are assessed for their biomass yields per hectare, the biochemical methane potential of their silages and their biomethane yields per hectare. Factors that influence these parameters are also investigated to find the best energy crops alternative to maize when harvested for biomethanation.

Abstract

The biomethane yield of various energy crops, selected among potential alternatives to maize in the Greater Region, was assessed. The biomass yield, the volatile solids (VS) content and the biochemical methane potential (BMP) were measured to calculate the biomethane yield per hectare of all plant species. For all species, the dry matter biomass yield and the VS content were the main factors that influence, respectively, the biomethane yield and the BMP. Both values were predicted with good accuracy by linear regressions using the biomass yield and the VS as independent variable. The perennial crop miscanthus appeared to be the most promising alternative to maize when harvested as green matter in autumn and ensiled. Miscanthus reached a measured biomethane yield of $5.5 \pm 1\ 10^3\ m^3.ha^{-1}$ during the second year after the establishment, as compared to $5.3 \pm 1\ 10^3\ m^3.ha^{-1}$ for maize under similar crop conditions.

Keywords: biochemical methane potential (BMP), lignocellulose, anaerobic digestion, biomethanation, energy crops, renewable energy.

1. Introduction

Along with the world population growth, the response to the increasing energy demand is a major challenge for humanity. The anaerobic digestion process appears to have a high potential to contribute to sustainable energy production. This bioprocess is one of the most advanced option to convert fermentable biomass, including the organic fraction of municipal solid waste, kitchen wastes, green wastes, aquatic biomass and dedicated energy crops (Nallathambi Gunaseelan, 1997), into a multipurpose fuel (CH_4) and fertilizers readily available to plant production systems (Möller & Stinner, 2010), while reducing greenhouse gas emissions, as compared to fossil fuels (Uusitalo et al., 2014).

Among the broad variety of substrates suitable for anaerobic digestion (Raposo et al., 2012), energy crops were extensively investigated, especially for their use in co-digestion agricultural biogas plants, together with animal effluents (Weiland, 2009). Whereas using only agricultural by- and co-products may limit the feeding of an anaerobic digester over the year, using arable land to produce biomass for an energy purpose is considered as an environmentally-friendly strategy if sustainability criteria are reached (Hanegraaf et al., 1998).

Various energy crops have been tested for anaerobic digestion (Bauer et al., 2009). Ideally, energy crops should offer a high dry biomass yield at a low cost, a composition with the least contaminants such as soil and should require low nutrient and energy inputs (McKendry, 2002a). It should also offer a low sensitivity to pest and a good soil cover, while not decreasing the biodiversity. In practice, maize is currently the most used energy crop for biomethanation because of its high biomass yield, good conversion rate into methane and easy storage as silage. Thus, the biomethane yield of maize silages has been widely assessed (Herrmann & Rath, 2012; Mayer et al., 2014). Its energy and CO_2 balances are quite favourable (Gerin et al., 2008). However, even if efforts are made to reduce the negative impacts of maize cropping such as soil erosion, soil compaction, low biodiversity, nutrient leakages into surface and groundwater, and pesticide pollution of soil and water (European Environment Agency, 2006), other plant species are investigated to substitute this crop as a source of biomass and to reach higher sustainability criteria.

The main objective of the present study is to measure and assess the biomethane yield of other plant species, including hemp, immature rye, miscanthus, sorghum, spelt, sunflower, switchgrass and tall fescue, that can potentially address some of environmental issues and could advantageously displace maize as a major substrate for biomethane production. The energy potential of these plant species was assessed for the Greater Region (the area including Saarland and Rhineland-Palatinate in Germany, Lorraine in France, the Luxembourg territory and Wallonia in Belgium). Additionally, factors such as the harvest time, the biomass yield, the volatile solids (VS) content and the anaerobic digestibility were analysed to identify their influence on the biomethane yield of these various plant species.

2. Materials and methods

2.1. Plant material

Annual and perennial plants studied for their biomethane yield are presented in Table 7.1. Hemp (*Cannabis sativa* L.), rye (*Secale cereal* L.), maize (*Zea mays* L.), miscanthus (*Miscanthus x giganteus* J.M. Greef & Deuter ex Hodk. & Renvoize), sorghum (*Sorghum bicolor* (L.) Moench), spelt (*Triticum aestivum* L. ssp. *Spelta* (L.) Thell.), sunflower (*Helianthus annuus* L.), switchgrass (*Panicum virgatum* L.) and tall fescue (*Festuca arundinacea* Schreb.), were produced in 2009, 2010 and 2011 at Gerbéviller (France), Mötsch (Germany), Libramont, Gembloux, and Tinlot (Belgium). The energy crops were thus grown under various agro-climatic conditions, including various fertilization schemes, in order to induce variability within the sample set and assess the various crop potentials in a way that is representative for the Greater Region. All plant species were harvested once a year, except tall fescue that was harvested three times per year. The crops were grown in 9 to 24 m^2 plots. The total aerial biomass was harvested and chopped at 10 cm above ground with a Haldrup M-65 harvester. In case of lodging, the biomass was harvested manually. Maize (additional 379 samples) that was previously assessed (Mayer et al., 2014) was included as a reference energy crop.

Since three ways of conversion of the biomass into energy (bioethanol, biomethanation and combustion) were envisaged in the ENERBIOM research project (ENERBIOM, 2012), some species were cropped or harvested in specific

ways. Indeed, grains (targeted for food) were separated from straw (biomass for energy) at the harvest of spelt. Rye was harvested as an immature cereal for silage production. Some maize and sorghum (18 and 9 samples respectively) were harvested at the end of the winter in March, in order to produce biomass with low moisture content suitable for combustion. In addition, a specific field trial that aimed at comparing maize, sorghum and miscanthus was conducted at Tinlot (Belgium) during the 2009-2011 cropping seasons. Such cropping design was aimed to create a large sample set of various biomasses to be tested for biomethanation. The specific influence of the harvest date, the cropping environment or the fertilization scheme on the biomethane yield was not targeted in the present study. All detailss about the agronomic trials can be found in the ENERBIOM final report (ENERBIOM, 2012).

The crude matter biomass yield ($biomass_{CM}$ yield in $t.ha^{-1}$) of each sample was measured at harvest before carrying a sample to the laboratory. Each sample was then packed in plastic bags under vacuum to allow a silaging process and storage. In case of gas production due to the silaging process, bags were opened and resealed under vacuum (Mayer et al., 2014). If no gas was produced (no silaging process was observed for spelt) or after the silaging process period (2-3 weeks at room temperature to reach stable and preserved silage), all samples were stored at room temperature in vacuum sealed bags until laboratory analysis.

Total solids content in the wet silages (TS) were measured after a drying step in an oven at 105°C for 24h, and volatile solids content (VS) in the wet silages was quantified subsequently after combustion in a furnace at 550°C for 6h.

2.2. BMP measurements

The biomethane produced by the silages was measured according to the VDI 4630 standard, with the method described previously (Mayer et al., 2014). The main parameters that characterize the BMP assays are summarized in Table 7.1, as recommended by Raposo et al. (2012). Briefly, the 2L total capacity batch anaerobic digesters were filled with the crop samples to be analysed individually and an inoculum collected from the anaerobic digester of a wastewater treatment plant. The inoculum was collected from a mesophilic anaerobic digester of the municipal wastewater treatment plant of Schifflange (SIVEC, Luxembourg). As recommended

by Angelidaki et al. (2009), the inoculum was incubated at 37°C for four days to decrease the endogenous biogas production.

An inoculum to substrate ratio of 2 (VS basis) was targeted at the start-up of the anaerobic digestion. The digesters were kept in water baths at constant mesophilic temperature (37°C) during batch assays. The produced biogas was cooled down in the exit tubing to condense water vapour (6°C). The biogas was collected into 10L gas bags and regularly measured on a daily basis during the first week, then once a week for the rest of the anaerobic digestion period. Biogas measurements consisted in volume quantification with a wet drum-type gas meter (TG05 wet-type, Ritter) and in composition analysis for methane and carbon dioxide, with specific infrared sensors (Dynament, UK). The biomethane volumes were normalised (273 K, 1013 hPa) according to the temperature and pressure conditions for each measurement. The measurements were then cumulated to quantify the BMP of the silage samples on a crude matter basis (BMP_{CM}). The endogenous biomethane production of the inoculum was measured in batch assays (triplicates) involving the inoculum alone. Microcrystalline cellulose was used as a control substrate in each series to check the inoculum activity. The BMP result of each experiment was validated when the BMP of microcrystalline cellulose digested simultaneously was in agreement with its expected biogas potential.

For each silage sample (a total of 693 silages without maize samples), a single digestion test was carried out, except for maize which was analysed in triplicates, whereas all the field replicates were analysed individually (3-16 replicates).

Table 7.1. Conditions used for the biochemical methane potential (BMP) assays.

Parameters	Value
Inoculum	
Origin	MWTP (Schifflange, Luxembourg), mesophilic anaerobic digester
Number of batch campaigns	38
Total solids	2.6 ± 0.7 %CM
Volatile solids	1.4 ± 0.4 %CM
Activity	Checked with microcrystalline cellulose
Degassing period prior to assays	4 days at 37°C
Control substrate	
Type	Microcrystalline cellulose
Total solids	96.2 %CM
Volatile solids	96.2 %CM
Amount and concentration at start-up of the experiment	10 gCM and 6 gVS.kg Inoculum^{-1}
BMP	367±15 mL.gVS^{-1}
Substrates	
Type	Energy crop silages and post-winter harvests
State	Wet
Total solids (%CM)	33.8 ± 17.7
Volatile solids (%CM)	31.4 ± 17.1
Experimental conditions	
Replicates	1 or 3 for maize
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 to 56 days
Headspace gas	No flushing at start-up
pH/alkalinity adjustment	No adjustment
Mineral medium	No mineral medium added
ISR	2.55 ± 1.04

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

2.3. Data processing and analysis

The biomass yield and the BMP on a VS basis ($biomass_{VS}$ yield and BMP_{VS}) were calculated using (*i*) the measured $biomass_{CM}$ yield of the wet sample in the field, (*ii*) the measured BMP_{CM} of the wet silage, and (*iii*) the measured VS content of the wet silage. The biomethane yield per hectare was calculated from the $biomass_{CM}$ yield and the BMP_{CM} (Mayer et al., 2014).

For spelt treated as two separated harvests (grain and straw), the whole plant VS, BMP, biomass yields and biomethane yield were calculated by adding the contribution of grains to the one of straw.

For tall fescue, the biomass yields (both $biomass_{CM}$ yield and $biomass_{VS}$ yield) and the biomethane yield of the three harvest dates were cumulated to obtain the annual yields.

Comparison of the means was carried out with SPSS, version 19 (SPSS Inc., 2010). The general linear model (GLM) procedure was used after assessing the normality of the distributions (Shapiro-Wilk's test) and the homoscedasticity (Levene's test). The Tukey or the T3-Dunnet post-hoc tests were carried out to compare means, depending of the homogeneity of the variances. An α-risk of 0.05 was used as the significant probability level for all statistical tests.

First-order linear regressions and non-linear regressions were also carried out with SPSS, version 19 (SPSS Inc., 2010) to determine the slope, the offset, the coefficient of determination (R^2) and the standard error of estimates (SEE). The same software was used to model the BMP_{VS} as a function of the VS content [$BMP_{VS} = a + (b/VS)$].

Non-linear curve fitting of points representing biomethane production over the digestion period were carried out for crops (maize, miscanthus, hemp, spelt straw, and switchgrass) showing incomplete digestion, using a 3-parameters logistic function (Groot, Cone, Williams, Debersaques, & Lantinga, 1996) with SigmaPlot 12.5 (Systat Software, 2011), to define the asymptotic value of biomethane production.

3. Results and discussion

3.1. Energy crops alternatives to maize

The measured biomass yields and BMP of the various energy crops are presented in Figure 7.1, together with the calculated biomethane yields per hectare. For most crops, the TS and VS contents in the wet biomass showed similar values. It was the case for maize (32 and 30 %CM), post-winter sorghum (30 and 28 %CM), immature rye (18 and 17 %CM), miscanthus (42 and 40 %CM), sorghum (21 and 19 %CM), sunflower (22 and 19 %CM), post-winter maize (70 and 67 %CM), and tall fescue (26 and 23 %CM) (Table 7.2). Thus, these biomasses showed a low content in ash. The TS and VS contents were less alike for hemp (45 and 40 %CM), switchgrass (63 and 58 %CM), spelt grains (86 and 81 %CM) and spelt straw (91 and 85 %CM), these crops showing a content in ash of about 5 %CM.

Table 7.2. Plant material, cropping details, number of samples analysed, total solids (TS) and volatile solids (VS) contents.

Plant species	Culture	Sowing or planting period	Harvest period	Samples	TS (%CM)	VS (%CM)
Hemp	Annual	Spring	Early autumn	4	44.8 ± 2.9	40.3 ± 3.2
Immature rye	Annual	Autumn	Early spring	28	18.1 ± 1.6	16.5 ± 1.6
Maize	Annual	Spring	Early autumn	491	32.4 ± 9.7	30.0 ± 6.4
Maize (post winter)	Annual	Spring	Late winter	18	69.8 ± 4.8	67.1 ± 8.0
Miscanthus	Perennial	Early spring	Early autumn	30	41.9 ± 3.5	40.2 ± 3.6
Sorghum	Annual	Spring	Early autumn	65	21.1 ± 4.5	19.4 ± 4.6
Sorghum (post-winter)	Annual	Spring	Late winter	9	30.3 ± 2.6	27.5 ± 2.8
Spelt (grain and straw)	Annual	Autumn	Summer	37	87.4± 4.3	82.8 ± 3.5
Sunflower	Annual	Spring	Early autumn	12	22.0 ± 3.0	19.0 ± 2.6
Switchgrass	Perennial	Mid-spring	Early autumn	27	62.8 ± 12.2	58.3 ± 11.4
Tall fescue	Perennial	Early spring or end summer	Mid spring Mid-summer Mid-autumn	426	26.3 ± 6.2	23.0 ± 5.5

Mean ± standard deviation of TS and VS are expressed as percentage of crude matter (CM).

The biomass production and the BMP were highly variable between the various plant species, but also between field replicates of each plant species. The variability of both parameters, biomass yield and BMP, resulted in a wide range of biomethane yield for each plant species. When converting crops into energy, the choice of the plant species influences the produced biomethane yield. Among the energy crops assessed in the present paper, maize harvested before the winter had the highest mean biomethane yield (6934 ± 1850 $m^3.ha^{-1}$), followed by miscanthus harvested before the winter (4468 ± 1265 $m^3.ha^{-1}$) and sorghum harvested before the winter (4332 ± 1175 $m^3.ha^{-1}$). Based on this large assessment in the Greater Region, these two plant species were the best alternatives to maize, among the assessed biomasses, to maximize the biomethane yield per hectare of cropped area.

The harvest date was chosen to correspond to a typical harvest of the crop, except for miscanthus, sorghum and maize where a winter harvest is unusual. The highest biomethane yields of the plant species may not be reached for the samples harvest in the present study. Such a study is a first step before carefully assessing the effect of the harvest date and the maturity of the plant on the biomethane yield.

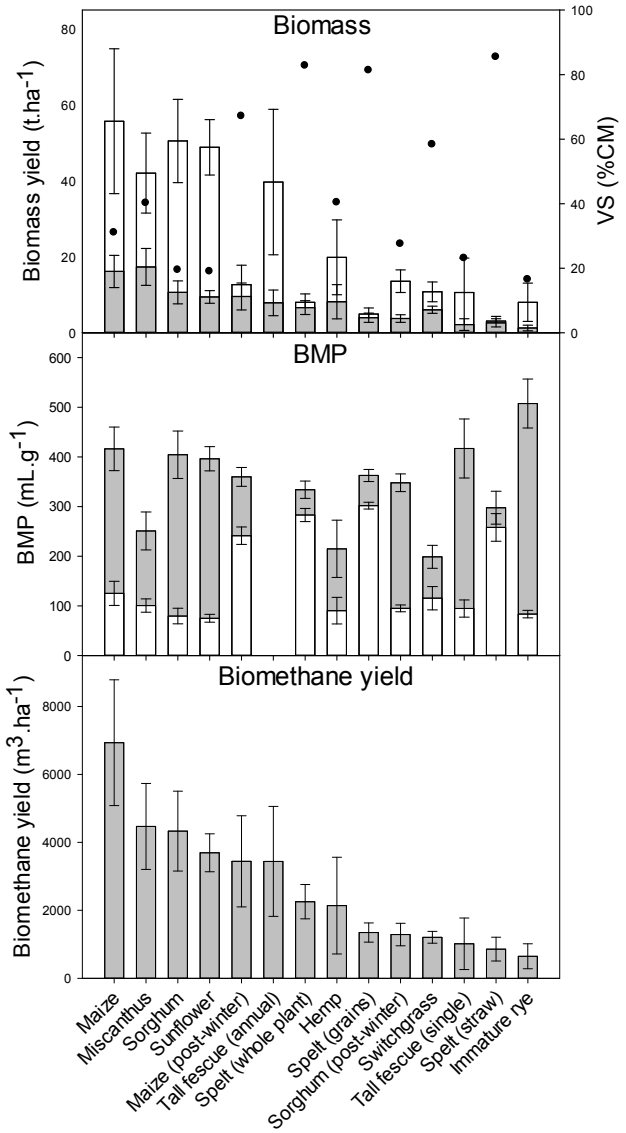

Figure 7.1. Influence of crop species on biomass yields and volatile solids (VS) content (top), biochemical methane potentials (BMPs) (middle) and biomethane yield per hectare (bottom). The VS content is indicated by black dots. Biomass and biomethane yields are annual yields per cropped area, except for "Tall fescue (single)", which represent a single, and not cumulated, harvest. White bars represent biomass yields and BMP on a crude matter (CM) basis and grey bars represent biomass yields and BMP on a VS basis. White bars and grey bars are overlaid and not cumulated bars. Error intervals represent the standard deviations of all field replicates tested for each crop.

Comparison between miscanthus, sorghum and maize

The biomass yield, BMPs and biomethane yield of the specific trial where miscanthus, maize and sorghum were cropped in the same field over a two to three years period (maize and sorghum in 2009-2010, miscanthus in 2009-2011) are shown in Figure 7.2. This is the first report on the biomethane yield per unit of cropped area of green miscanthus harvested in autumn and processed as silage. The $biomass_{CM}$ yield of miscanthus increased over the three consecutive years after plantation of rhizomes. After the first establishment year, its $biomass_{VS}$ yield was higher than those of maize and sorghum, owing to its high VS content at harvest. However the BMP_{VS} of miscanthus were significantly lower than those of maize and sorghum and did not show a clear tendency to increase or decrease with the age of the crop. Excluding miscanthus plantation year (2009), the biomethane yields of the three plant species were not significantly different. However, miscanthus reached a measured biomethane yield of 5508 ± 1016 $m^3.ha^{-1}$ during the second year after the establishment, as compared to 5322 ± 1044 $m^3.ha^{-1}$ for maize under similar crop conditions (Figure 7.2). According to this specific field trial, miscanthus harvested in autumn is the most promising alternative to maize for anaerobic digestion, because of its higher $biomass_{VS}$ yield, nevertheless counterweighted by a lower BMP_{VS}.

Miscanthus is usually harvested at the end of winter and is mainly recommended for combustion due to its low moisture content (Lewandowski & Heinz, 2003) and not for biomethanation (RMT Biomasse, 2009). However, low moisture content is not important for anaerobic digestion and an extended harvest window is thus available for miscanthus (Hayes, 2013). The $biomass_{VS}$ yield is higher before the winter due to the presence of the leaves, which are mainly lost during this period (Lewandowski & Heinz, 2003). An early harvest is then advised to reach high biomass yield and improve biorefinery yields (Hayes, 2013).

While miscanthus offers high biomass yields over its lifespan when harvested at the end of winter (Gauder et al., 2012; Caslin et al., 2011), the sustainability of an autumn harvest has yet to be assessed over a long period (15-20 years). Indeed, Godin et al., (2013b) pointed out that miscanthus harvested too early in autumn might not be able to translocate its nutrients to its rhizomes and a higher fertilization level would be needed. The detrimental effect of an early harvest in August was

pointed out elsewhere (Bayern Biogas Forum, 2010). Such problems for harvest in October, when plant metabolites and nutrients are potentially translocated to the rhizomes, were neither observed nor reported so far.

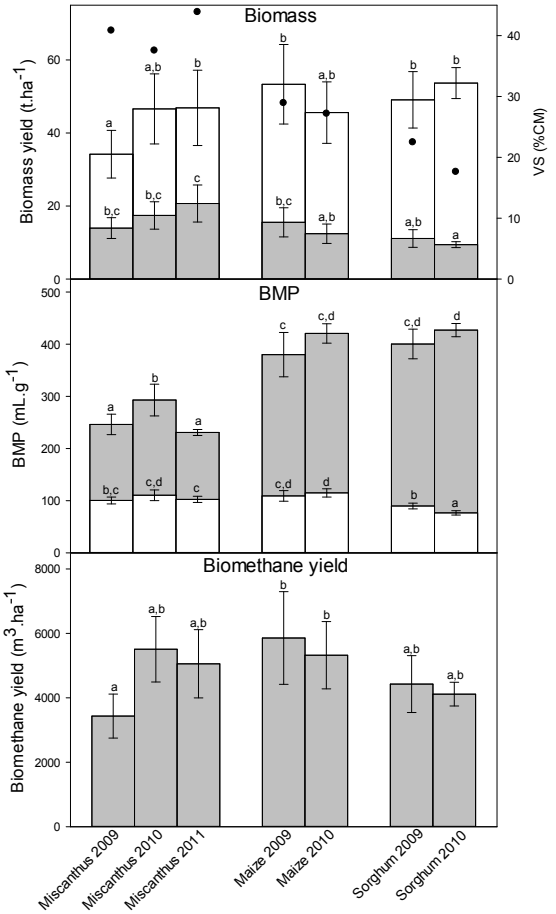

Figure 7.2. Influence of the harvest years (2009, 2010 and 2011) on biomass yields and volatile solids (VS) (top), biochemical methane potentials (BMPs) (middle) and biomethane yield per hectare (bottom) of miscanthus, maize and sorghum. The VS content is indicated by black dots. White bars represent biomass yields and BMP on a crude matter (CM) basis and grey bars represent biomass yield and BMP on a VS basis. White bars and grey bars are overlaid and not cumulated bars. Error intervals represent the standard deviations of all field replicates tested for each condition. Bars holding different letters for a single parameter differ significantly ($p < 0.05$).

Different methane production kinetics were observed between maize and miscanthus (Figure 7.3). Over the digestion period in batch assays, the biomethane production was faster for maize than for miscanthus. An asymptotic plateau was reached for maize samples at the end of the digestion (42 days), whereas the tilted profile of the biomethane production curve of miscanthus indicated that the conversion of biomass to biomethane was still on-going at this time. A curve-fitting of the time points according to a 3-parameters sigmoidal model (Groot et al., 1996) showed that for maize the calculated asymptotic BMP value (115 ± 7 mL.gCM^{-1}) was similar to the measured value (115 ± 8 mL.gCM^{-1}), whereas for miscanthus, the calculated asymptotic BMP value (166 ± 20 mL.gCM^{-1}) was higher than the measured BMP (110 ± 10 mL.gCM^{-1}).

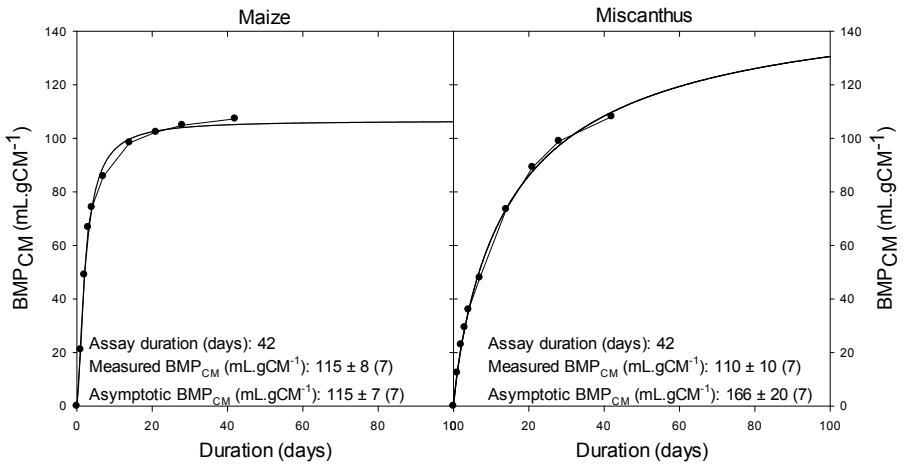

Figure 7.3. Comparison of CH4 production kinetics during biochemical methane potential (BMP) assays between maize and miscanthus silages. A 3-parameter logistic regression (Groot, 1996) was used to fit a curve on the cumulated biomethane production over time and determine the asymptotic BMP value on a crude matter (CM) basis (BMP_{CM}). The results are expressed as average ± standard deviation. The number of samples is indicated in brackets.

The chemical composition of miscanthus, maize and sorghum samples of the present study was characterized in a previous paper (Godin et al., 2013a). Miscanthus presents higher contents of structural compounds compared to maize and sorghum. The slow methane production kinetic of miscanthus, as compared to maize, can be

explained by the lignocellulosic composition of the biomass. Cellulose fibres are tightly linked to other polymers, such as hemicellulose and lignin and are difficult to degrade (Tsavkelova & Netrusov, 2012). Consequently, it is highly probable that the BMP and biomethane yield per cropped area of miscanthus would be higher if this substrate was exposed to long digestion time. Such long digestion times (longer than 100 days) are commonly observed in agricultural anaerobic digestion plants (Linke et al., 2013).

Some authors (Triolo et al., 2011; Buffiere et al., 2006) reported that the lignocellulosic fraction of biomass is negatively correlated with the BMP_{VS} and that lignin is the principal substance that limit the conversion of VS to methane. The high amount of lignocellulosic components within the biochemical composition of the biomass induces a slow methane production kinetic than can explain the lower BMP_{VS} measured after 42 days of digestion for miscanthus, as compared to maize and sorghum.

Miscanthus, a perennial plant which can thrive up to 15-20 years, looks like a promising alternative to the annual cropping of maize for biomethane production, if harvested before the winter.

Influence of wintering on biomethane yield

The effect of a longer cropping period on the biomethane yield was assessed for some maize and sorghum plots that were left in the field over the winter period (Figure 7.1). Biomethane yields of these plots (maize: 3441 ± 1341 $m^3.ha^{-1}$, sorghum: 1287 ± 330 $m^3.ha^{-1}$) were significantly ($p < 0.05$) lower than those obtained for the plots harvested in autumn. Maize and sorghum harvested after the winter have a higher VS content but a lower $biomass_{VS}$ yield than those harvested in autumn. The anaerobic digestibility of the post-winter sorghum was significantly lower ($p < 0.05$) than that of autumn green mater. In case of maize, the lower BMP_{VS} for the post-winter harvest was not significant ($p = 0.21$). The biomass composition at the end of the winter showed higher structural compounds (hemicellulose, cellulose and lignin) and less soluble sugars and proteins (Godin et al., 2013b). A higher proportion of starch was found in maize after winter. Leaves were lost during the winter for both plant species. The solubilisation and the leaching of the non-structural components during the winter (Cadoux et al., 2009)

can also explain the loss of digestible material. The stalks with cobs and some leaves were harvested in the case of maize whereas sorghum stalks suffered from lodging and were found on the ground. The physiological changes and the different biochemical composition of biomass before and after the winter can explain the difference of biomass yield and BMP.

Miscanthus was not harvested after the winter in the present study. However, the BMP_{VS} of miscanthus harvested after the winter was described as low (84 $mL.gVS^{-1}$) and the use of steam-explosion was suggested to improve the biomethane production of miscanthus (Menardo et al., 2012). The BMP_{VS} of miscanthus harvested before the winter and measured in the present study (higher than 200 $mL.gVS^{-1}$, Figure 7.2) was higher than that reported by Menardo for post-winter harvest. As for maize and sorghum, an increase in the fibre fraction (cellulose, hemicellulose and lignin), balanced with a decrease in total soluble sugars and proteins, impacted negatively the miscanthus digestibility from October to April (Godin et al., 2013a). It can be hypothesized that an earlier harvest of miscanthus than what was performed in the present trials could provide miscanthus with better digestibility.

Switchgrass

Similarly to miscanthus, switchgrass is usually harvested after the winter to be valorised through combustion. In the present study, switchgrass was harvested early, in autumn. As illustrated in Figure 7.1, switchgrass provided low biomass yields and the lowest BMP among the plant species assessed, resulting in very low biomethane yields. The BMP_{VS} were similar to the ones reported by Massé (2010). Godin et al., (2013b) observed that switchgrass samples harvested in autumn have no soluble sugars and a similar fibre part as compared to miscanthus harvested in autumn. The low BMP_{VS} of switchgrass can be explained by the high proportion of recalcitrant lignocellulosic fibres.

The $biomass_{VS}$ yields of switchgrass observed in the present trial were lower than in other trials (Massé et al., 2010). Switchgrass was sown in 2009 and showed a low germination potential of 33% compensated by an adjusted sowing rate (ENERBIOM, 2012). Moreover, herbicide application at the beginning of the trial to control weeds was not as efficient as in other crop. The establishment and biomass

production of switchgrass was then poor. Therefore, the biomass yield of switchgrass presented here is not representative of the full potential of this plant for its use as an energy crop. Massé et al. (2010) reported a maximum annual dry biomass yield of 12 $t.ha^{-1}$. However the biomethane yield of switchgrass, which could result from such a biomass yield and from its digestibility measured here, is not competitive as compared to the measured biomethane yield of maize silages.

3.2. Anaerobic digestibility of plant materials

The influence of the VS content of various ensiled plant materials on the measured BMP is presented in Figure 7.4 and in Table 7.3. Samples of miscanthus, switchgrass, hemp and spelt straw have lower BMP_{CM} than other samples included in the main linear scatterplot (Figure 7.4). Since the VS content is responsible for the methane production, the BMP_{CM} was modelled from the VS content according to a linear function crossing the axis origins. When excluding miscanthus, switchgrass, hemp, spelt straw and spelt whole plant samples, the BMP_{CM} increases linearly with VS content according to a mean slope of 395 ± 2 $mL_{CH4}.gVS^{-1}$ (R^2 of 0.89) (Figure 7.4, top). For most of the tested biomasses, the organic matter conversion yield seems quite constant. Such a value ca be compared to the theoretical conversion factor of carbohydrates in methane of 395 $mL_{CH4}.gVS^{-1}$ (Baserga, 1998). It is in accordance with the fact that plant materials are mainly made of structural carbohydrates (fibres) and also non-structural carbohydrates (starch) for maize specificaly.

Miscanthus, switchgrass, hemp and spelt straw have lower digestibilities than other plant species (Figure 7.4). Moreover, the biomethane production curves over the digestion period of those plant species (not shown) were very similar to that of miscanthus (Figure 7.3) while the other samples were similar to maize. Hemp, spelt straw and switchgrass have measured BMP_{CM} of 90 ± 27, 258 ± 28 and 117 ± 24 $mL.gCM^{-1}$ respectively, whereas those biomasses have higher asymptotic BMP_{CM} of 100 ± 25, 332 ± 56 and 153 ± 21 $mL.gCM^{-1}$ respectively, calculated from the curve fittings. Those biomasses have high levels of structural compounds (lignocellulosic fibres) within their biochemical composition, as compared to tall fescue, immature rye and maize, which were characterised by higher amount of total soluble sugars, proteins and starch (Godin et al., 2013a). The conversion of the VS

into methane was thus affected by the VS composition and its ability to be digested. For such plant species with high fibrous content, the digestion of the VS content was slow and not fully completed after 42 days of anaerobic digestion. Longer digestion period should be recommended to assess the BMP of biomass characterised by high fibre fractions.

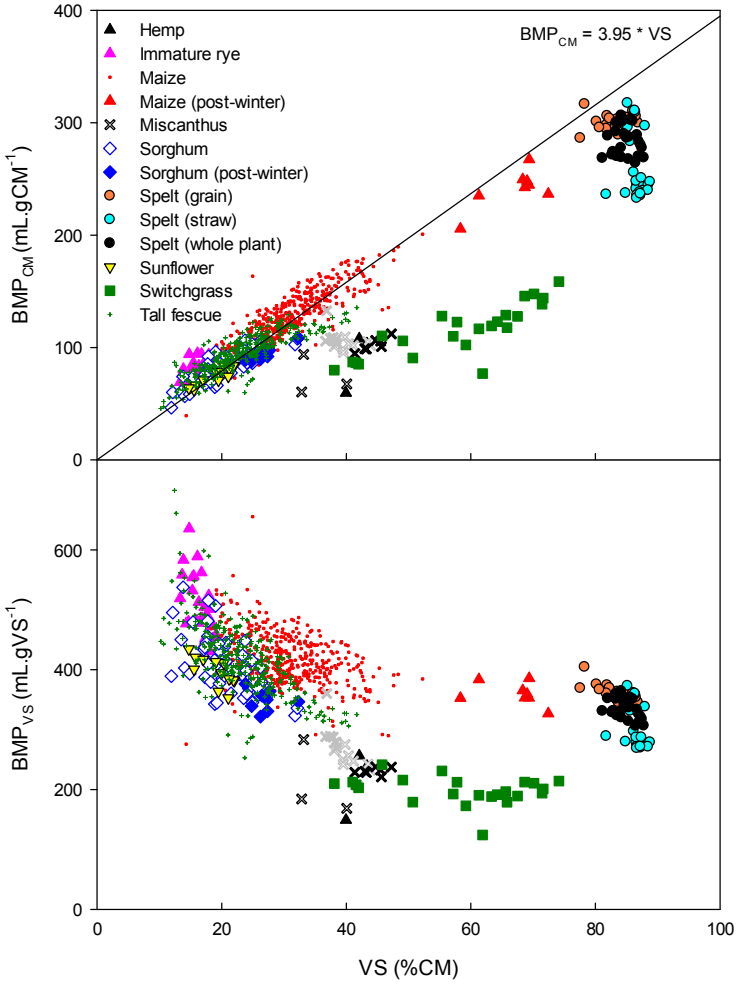

Figure 7.4. Influence of the volatile solids (VS) content of silage on the biochemical methane potential on a crude matter basis (BMP_{CM}, top) and on a volatile solids content basis (BMP_{VS}, bottom). Miscanthus samples (oblique crosses) were grouped and differentiated according to the harvest year (2009: open, 2010: grey, 2011: black).

Table 7.3. Parameters of the regressions between the volatile solids content (VS) and (A, linear) the biochemical methane potential on a crude matter basis (BMP_{CM}) or (B, non-linear) on a volatile solids content basis (BMP_{VS}).

(A) BMP_{CM} = Slope * VS + Intercept

Plant species	N	Slope	Intercept	R^2	SEE
All	972	2.7	36	0.80	21
Hemp	3	14.1	-499	0.81	17
Immature rye	28	2.3	45	0.25	7
Maize	459	3.3	25	0.76	12
Maize (post-winter)	8	2.7	64	0.51	13
Miscanthus	26	0.8	67	0.05	14
Sorghum	50	2.7	28	0.71	8
Sorghum (post-winter)	8	2.5	27	0.86	3
Spelt (grain)	23	0.8	233	0.10	7
Spelt (straw)	25	-.3.0	514	0.02	28
Spelt (whole plant)	23	-0.2	298	0.01	14
Sunflower	12	2.8	22	0.82	4
Switchgrass	23	1.7	13	0.68	14
Tall fescue (single)	307	2.5	37	0.72	9

(B) BMP_{VS} = a + (b/VS)

Plant species	N	a	b	a + (b/100)	R^2	SEE
All	995	291	2915	320	0.35	56
Hemp	3	1439	51078	1949	0.77	39
Immature rye	28	250	4204	292	0.30	42
Maize	459	348	1950	367	0.12	41
Maize (post-winter)	8	285	5030	335	0.09	19
Miscanthus	26	106	5786	164	0.12	37
Sorghum	50	299	2170	321	0.28	41
Sorghum (post-winter)	8	302	1233	314	0.05	18
Spelt (grain)	23	80	23549	315	0.55	8
Spelt (straw)	25	-258	48170	224	0.08	33
Spelt (whole plant)	23	-19	29905	280	0.19	16
Sunflower	12	279	2196	301	0.51	18
Switchgrass	23	166	1815	184	0.09	23
Tall fescue (single)	307	274	3101	305	0.45	44

N: number of samples, R^2: coefficient of determination, SEE: standard error of estimates.

The BMP_{VS} tend to decrease when the VS content increases (Figure 7.4 and Table 7.3, bottom). Anaerobic biodegradation of the organic material is more advanced for plant with a low VS content than for plant species with a high VS content. The VS content increased over the cropping period and can be related to the maturity of the plant for tall fescue (data not shown) and maize (Mayer et al., 2014). It has been shown that the structural compounds of most plant species increase with maturity (Godin et al., 2013b). The VS content is thus correlated with the biochemical composition of the plant, especially the less digestible fraction (recalcitrant fibres). For most biomasses, the digestibility decrease can be related with increasing maturity and the corresponding increase of structural components in the biomass.
Grains of spelt are characterized by a high amount of starch in the endosperm, which is surrounded by outer layers showing a composition similar to straw (Shewry et al., 2013). Such a composition explains the higher digestibility of grains compared to straw, at a high VS content.

3.3. Prediction of the BMP and the biomethane yield

BMP

Linear regressions between the VS content and the BMP were determined for all and each plant species (Table 7.3 and Figure 7.4). Owing to the good correlation and low SEE observed for all plant species tested together (R^2 = 0.80, SEE = 21 $mL.gCM^{-1}$), the VS content of a biomass can be used to predict its BMP_{CM}. However, this result is mostly valid for maize and tall fescue since it is highly influenced by their large number of samples (maize: N = 459, and tall fescue: N = 307), as compared to other plant species. The samples were also mostly distributed in a range where the VS content is less than 45 %CM. Such a distribution gives better prediction results for plant species having VS in this range. Nevertheless, the prediction of BMP_{CM} using the VS content showed good results for most plant species assessed individually, as shown by the SEE values. The low coefficient of determination for miscanthus, spelt and immature rye indicates that the BMP_{CM} was less influenced by their VS content than for the other biomasses. Such results can be explained by a low variability of the digestibility between the biomasses as seen above. Relationships specific to a

plant can also be used, paying attention to the regression coefficient, sample number and the SEE, that are variable according to the plant species (Table 7.3).

Since the BMP_{CM} can be modeled with a first-order linear regression using the VS content as predictor (BMP_{CM} = a*VS + b) and considering that the BMP_{VS} is the ratio between the BMP_{CM} and the VS content (BMP_{VS} = BMP_{CM}/VS), the BMP_{VS} can be modeled as BMP_{VS} = a + (b/VS) using the VS content as the only predictor. With such a model, the asymptotic value equal to a + (b/100) corresponds to the minimum digestibility of the plant species.

However, the VS content alone was not a good predictor to measure the BMP_{VS}, according to low R^2 values (Table 7.3 and Figure 7.4). Moreover, regressions for hemp or spelt are not realistic due to the low number of samples or to the data distribution that does not fit to such model.

Other mathematical models using the VS as a predictor have been tested to predict the BMP_{VS}, but they did not improve the prediction results (data not shown). Other parameters than the VS content are thus required to predict adequately the BMP_{VS} of energy crops. Some authors succeeded to characterize BMP_{VS} as the result of linear relations between the different biochemical fractions for different biomass (Nallathambi Gunaseelan, 2007; Amon et al., 2007; Raju et al., 2011). Such models allow for the prediction of the BMP_{VS} from biochemical analysis, with some uncertainty. Analysis of the VS composition would thus be needed to predict accurately the BMP_{VS} of biomasses.

Biomethane yield

The influence of the biomass yields (both $biomass_{CM}$ and $biomass_{VS}$ yields) on the biomethane yield per hectare is presented in Figure 7.5 and in Table 7.4. A relationship can be observed between the $biomass_{CM}$ yields and the biomethane yield. The $biomass_{CM}$ yield variability explains 87% of the variability of the biomethane yield. Since the moisture contained in the biomass does not contribute to the production of methane, the influence of the $biomass_{VS}$ yield on the biomethane yield was also characterized. When considering all plant species together, the $biomass_{VS}$ yield explains a larger part (R^2 = 0.94) of the variability of the biomethane yield, as compared to the $biomass_{CM}$ yield.

Similarly to Figure 7.4, samples of miscanthus, switchgrass, spelt and hemp are observed below the main scatter plot of Figure 7.5B. The measured BMP_{VS} of these samples were lower than other plant species and can be explained by a slow anaerobic digestion that can be incomplete at the end of the batch assay, due to high recalcitrant fibre contents.

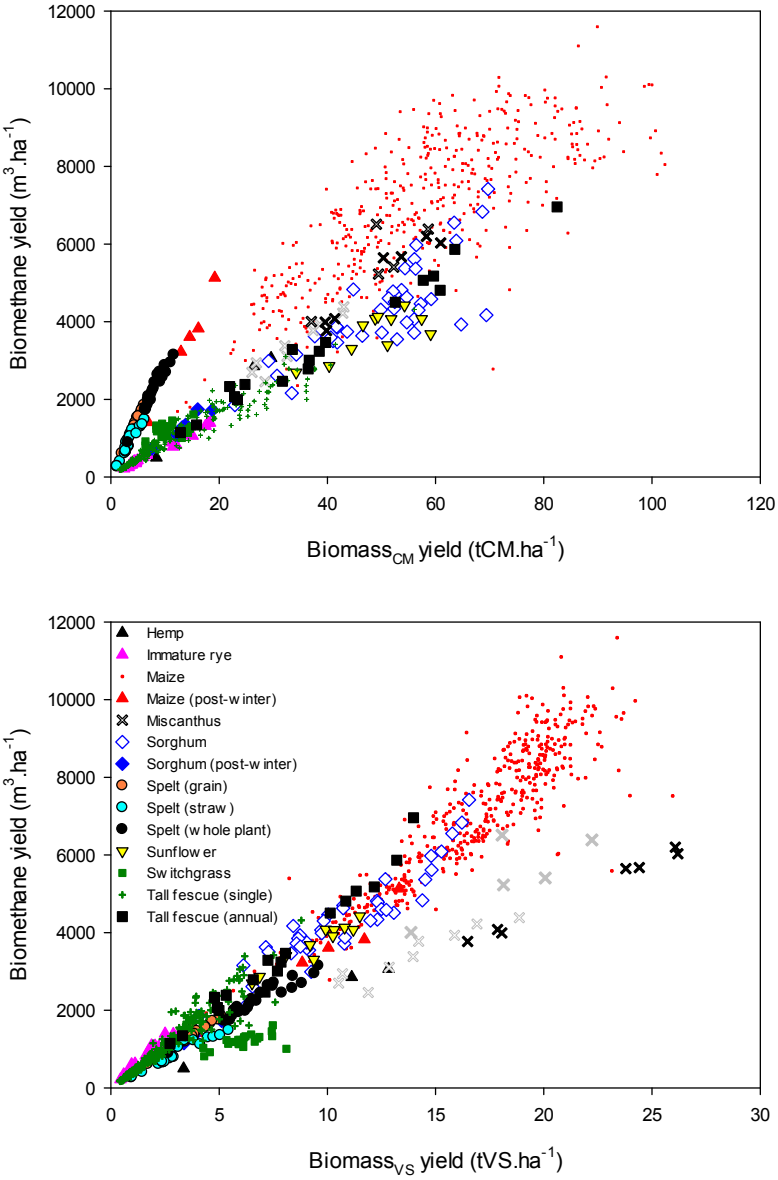

Figure 7.5. Influence of the biomass yield on a crude matter basis (biomass$_{CM}$ yield, top) and on a volatile solids content basis (biomass$_{VS}$ yield, bottom) on the biomethane yield per hectare for the various crops studied. Miscanthus samples (oblique crosses) were grouped and differentiated according to the harvest year (2009: open, 2010: grey, 2011: black).

Table 7.4. Parameters of the linear regressions between the biomethane yield and (A) the produced biomass yield on a crude matter basis ($biomass_{CM}$) or (B) on a volatile solids content basis ($biomass_{VS}$).

(A) Biomethane yield = Slope * $biomass_{CM}$ yield + Intercept

Plant species	N	Slope	Intercept	R^2	SEE
All	954	108	240	0.87	1099
Hemp	3	124	-529	0.99	130
Immature rye	28	72	67	0.98	50
Maize	459	80	2309	0.61	1134
Maize (post-winter)	5	291	-612	0.99	185
Miscanthus	21	115	-434	0.91	382
Sorghum	40	84	113	0.63	723
Sorghum (post-winter)	8	99	-53	0.92	110
Spelt (grain)	23	305	-15	0.99	30
Spelt (straw)	25	241	54	0.92	99
Spelt (whole plant)	23	269	105	0.95	115
Sunflower	11	57	889	0.56	389
Switchgrass	23	32	859	0.23	156
Tall fescue (single)	267	74	150	0.94	190
Tall fescue (annual)	18	84	107	0.98	237

(B) Biomethane yield = Slope * $biomass_{VS}$ yield + Intercept

Plant species	N	Slope	Intercept	R^2	SEE
All	912	410	-61	0.94	756
Hemp	3	281	-416	0.99	191
Immature rye	28	490	22	0.96	72
Maize	458	433	-277	0.83	743
Maize (post-winter)	5	371	-116	0.96	297
Miscanthus	21	234	340	0.78	612
Sorghum	40	357	496	0.86	440
Sorghum (post-winter)	8	314	133	0.99	38
Spelt (grain)	23	358	17	0.98	46
Spelt (straw)	25	278	55	0.92	102
Spelt (whole plant)	23	313	132	0.90	163
Sunflower	11	320	666	0.92	172
Switchgrass	23	112	520	0.40	138
Tall fescue (single)	267	442	-27	0.92	213
Tall fescue (annual)	18	473	-302	0.97	277

N: number of samples, R^2: coefficient of determination, SEE: standard error of estimate

Relationships between the biomass yield and the biomethane yield were further characterized specifically according to the plant species (Table 7.4). The lowest values of SEE for the local linear regressions showed that local assessments (plant species specific) describe better the influence of the biomass yield on the biomethane yield than the global linear regression (all plant species considered).

Linear regressions using only the $biomass_{VS}$ yield as independent variable allow good predictions of the biomethane yield of the various biomasses. Miscanthus ($R^2 = 0.78$) and switchgrass ($R^2 = 0.40$) show coefficient of determination lower than 0.80 for the linear regression between the $biomass_{VS}$ yield and the biomethane yield. The removal of only one outlier in each dataset increased the R^2 value to 0.89 and 0.73 for miscanthus and switchgrass respectively.

The amount of biomass, especially considered on a dry matter basis, produced in the field appears to be the main factor driving the biomethane yield. This conclusion has possibly to be adjusted by considering that the net energy output from the biomethanation of an energy crop decreases with increasing water content because of higher energy investment in harvest and transportation of undesirable water (Berglund & Börjesson, 2006). In this perspective, miscanthus could have a comparative advantage to maize thanks to its higher VS content.

Whereas the BMP was extensively assessed for various biomasses (Bauer et al., 2009; Amon et al., 2007; Raposo et al., 2012), the present paper shows that the anaerobic digestibility influences the biomethane yield but does not appear to be of prior importance to reach high biomethane yield per cropped area. The choice of an energy crop dedicated to biomethane production should thus be driven by the main criterion of $biomass_{VS}$ yield. However, the agricultural practices (annual vs perennial crops, planting vs sowing, single or multiple harvest per year, harvest and transport, water needs, pesticide use, etc…) which are specific to plant species must also be considered when assessing the energy and resource efficiency and the environmental benefit of a biomass-for-energy production system (Börjesson & Berglund, 2007).

4. Conclusion

Crops with high biomass yield should be preferred for biomethane production. The BMP is influenced by the plant species and it has low influence on the biomethane yield per hectare. Miscanthus silage harvested in autumn produces high and

competitive biomethane yield, as compared to maize. At the end of the batch assays, a conversion yield of 395 ± 2 mL.gVS^{-1} was observed for most plant biomasses, except for the most fibrous ones such as miscnathus that have a slower digestion kinetic. The VS content and the biomass$_{VS}$ yield.ha^{-1} provide sufficient information to estimate the BMP and biomethane yield of known plant species.

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Annexe

Cropping design used for biomass production of the ENERBIOM samples. Full description is available in the ENERBIOM final report (ENERBIOM, 2012).

Environment	Plant species	Sowing date	Harvest date
Libramont	Tall fescue	14/08/08	27/05/09
Libramont	Tall fescue	14/08/08	04/08/09
Libramont	Tall fescue	14/08/08	25/10/09
Libramont	Tall fescue	14/08/08	02/06/10
Libramont	Tall fescue	14/08/08	10/08/10
Libramont	Tall fescue	14/08/08	25/10/10
Libramont	Tall fescue	14/08/08	18/05/11
Libramont	Tall fescue	14/08/08	01/08/11
Libramont	Tall fescue	14/08/08	03/10/11
Libramont	Maize	04/05/10	29/10/10
Libramont	Immature rye	09/09/09	28/04/10
Gembloux	Tall fescue	30/08/08	19/05/09
Gembloux	Tall fescue	30/08/08	13/07/09
Gembloux	Tall fescue	30/08/08	21/10/09
Gembloux	Tall fescue	30/08/08	19/05/10
Gembloux	Tall fescue	30/08/08	06/07/10
Gembloux	Tall fescue	30/08/08	14/10/10
Gembloux	Tall fescue	30/08/08	09/05/11
Gembloux	Tall fescue	30/08/08	02/08/11
Gembloux	Tall fescue	30/08/08	03/10/11
Gembloux	Maize	05/05/10	15/10/10
Gembloux	Immature rye	09/09/09	27/04/10
Gembloux	Spelt (straw)	26/10/10	10/08/11
Gembloux	Spelt (grains)	26/10/10	10/08/11
Tinlot	Sorghum	21/05/09	08/10/09
Tinlot	Sorghum	21/05/09	09/03/10
Tinlot	Sorghum	04/06/10	14/10/10
Tinlot	Miscanthus	26/04/08	08/10/09
Tinlot	Miscanthus	26/04/08	14/10/10
Tinlot	Miscanthus	26/04/08	05/10/11
Tinlot	Switchgrass	29/04/08	08/10/09
Tinlot	Switchgrass	29/04/08	14/10/10
Tinlot	Maize	04/05/09	08/10/09
Tinlot	Maize	04/05/09	09/03/10
Tinlot	Maize	23/04/10	14/10/10
Tinlot	Maize	23/04/10	23/03/11
Tinlot	Spelt (straw)	27/10/09	12/08/10
Tinlot	Spelt (grains)	27/10/09	12/08/10

Mötsch	Tall fescue	06/04/09	17/08/09
Mötsch	Tall fescue	06/04/09	29/09/09
Mötsch	Tall fescue	06/04/09	27/05/10
Mötsch	Tall fescue	06/04/09	21/07/10
Mötsch	Tall fescue	06/04/09	23/09/10
Mötsch	Tall fescue	06/04/09	04/05/11
Mötsch	Tall fescue	06/04/09	11/07/11
Mötsch	Tall fescue	06/04/09	18/10/11
Mötsch	Maize	22/04/09	21/09/09
Mötsch	Spelt (straw)	15/10/09	07/08/10
Mötsch	Spelt (grains)	15/10/09	07/08/10
Gerbéviller	Sorghum	12/05/09	14/09/09
Gerbéviller	Sorghum	07/06/11	13/10/11
Gerbéviller	Tall fescue	12/05/09	19/08/09
Gerbéviller	Tall fescue	12/05/09	25/09/09
Gerbéviller	Tall fescue	12/05/09	07/06/10
Gerbéviller	Hemp	12/05/09	29/09/09

Highlights of Chapter 7

- Miscanthus harvested before the winter and maize produce the highest biomethane yields in the Greater Region.
- The biomass yield mainly influences the biomethane yield of energy crops.
- The BMP_{CM} can be predicted with the VS content of crude wet silages of energy crop.
- The anaerobic digestibility of most biomasses is similar.
- Plant species with high fibre content such as miscanthus are slowly digested.

Chapter 8

Fast prediction of the biochemical methane potential of energy crops with near infrared spectroscopy

Various energy crops have been produced and assessed for their BMP as wet ensiled substrates (Chapter 3 and 7). The VS content has been shown to provide useful information to estimate the BMP_{CM} of these energy crops (Chapter 8). However, the BMP_{VS} was shown to be different according to the plant species and their fibre content. For maize silages specifically, the biochemical composition, the BMP_{CM} of wet silages and of dry and ground powder were successfully predicted with models based on NIRS (Chapter 5 and 6). NIRS can thus also be investigated as a predictor of the BMP of various energy crops. **What is the ability of models based on NIRS to predict the BMP of various wet silages of energy crops?**

In the present chapter (Chapter 8), the prediction of the biochemical methane potential of various wet energy crop silages is specifically investigated with NIRS.

Abstract

The biochemical methane potential (BMP) of energy crops is measured with a time-consuming assay (digestion of at least one month). Near infrared spectroscopy (NIRS) was investigated as an alternative approach to predict quickly the BMP of energy crops. The volatile solids (VS) content and the BMP of crude silages of various plant species such as maize, tall fescue, sorghum, spelt, miscanthus, immature rye, switchgrass, sunflower and hemp were measured. The NIR spectra were acquired on crude silage samples and on dried and ground samples of the same silages. Both spectra types were used as predictors of the BMP of crude silages. NIRS measured on the crude samples successfully predicted the VS content and the BMP on a crude matter basis with a RMSECV of 2.7 %CM and 14 $mL.gCM^{-1}$, representing 8% and 12% of the respective means. Prediction of the BMP on a VS basis was less performing according to the RPDCV of 1.6, because of the narrow range of BMP_{VS} observed for the various crops tested, as compared to the accuracy of the BMP assay. NIRS could discriminate between high and low BMP_{VS}, similarly to the plant species. The repeatability of the BMP assay must be improved to predict more accurately the BMP_{VS} of various energy crops.

Keywords: biochemical methane potential, near infrared spectroscopy, energy crops, biomethanation, anaerobic digestion, biogas, biomass.

1. Introduction

Anaerobic digestion is considered as an environmental-friendly and competitive way to produce biomethane as a source of renewable energy (Demirbas, 2008). It reduces greenhouse gases emission and is a valorization way for organic wastes (Ward, 2008).

Agricultural products are a particularly suitable feedstock for biogas production. They consist mainly in animal effluents and plant co-products. Energy crops were developed to complement the feedstock of anaerobic digesters in order to increase the biomethane production. The biomethane yield per hectare of energy crops is the most important parameter considered by farmers to choose plant species, even if agricultural practices must be also thought (Bauer et al., 2009).

The biomethane yield of energy crops ($m^3_{CH4}.ha^{-1}$) is calculated from the biomass yield per hectare ($t_{biomass}.ha^{-1}$) and from the biochemical methane potential (BMP in $m^3_{CH4}.t^{-1}_{biomass}$).

The BMP is measured in small anaerobic digesters, under laboratory conditions. It requires dedicated material and is time-consuming, at least one month (Raposo et al., 2012). Such a time is too long for stakeholders in many cases to make a decision about the value of a substrate. Indirect measurements of the BMP have been investigated with the biochemical composition or near infrared spectroscopy (NIRS).

The influence of the biochemical fractions of energy crops on their BMP have been widely assessed and models that predict the BMP have been created (Amon et al., 2007; Nallathambi Gunaseelan, 2007; Schievano et al., 2008; Nallathambi Gunaseelan, 2009b; Schievano et al., 2009; Triolo et al., 2011; Rath et al., 2013). However, analyzing the biochemical composition still requires time and laboratory work to predict the BMP.

NIRS is a fast analytical technique that uses interaction between light and matter to characterize the composition of this matter. NIRS was successfully used to predict the BMP of various organic materials (Lesteur et al., 2011; Doublet et al., 2013) and specific biomasses like grass (Raju et al., 2011) or maize (Chapter 4). Moreover, NIRS was assessed to monitor important parameters of anaerobic digestion, such as volatile fatty acids, carried out in laboratory and in full-scale biogas plants (Jacobi et

al., 2009; Lomborg et al., 2009; Krapf et al., 2011; Stockl & Oechsner, 2012). In this context, NIRS appears as a useful technic for anaerobic digestion.

The BMP of various plant species used as energy crops were assessed in a previous article (Mayer et al., 2014). The objective of this paper is to assess the ability of NIRS to predict the BMP of all of these energy crops. The specific aim is to create a model based on NIR spectra of biomass to quickly predict the BMP with good accuracy, as compared to the reference method.

2. Material and methods

2.1. Biomass production

The biomass production was detailed by Mayer et al. (2014a; 2014b). In brief, hemp (*Cannabis sativa* L.), rye (*Secale cereal* L.), maize (*Zea mays* L.), miscanthus (*Miscanthus x giganteus* J.M. Greef & Deuter ex Hodk. & Renvoize), sorghum (*Sorghum bicolor* (L.) Moench), spelt (*Triticum aestivum* L. ssp. *Spelta* (L.) Thell.), sunflower (*Helianthus annuus* L.), switchgrass (*Panicum virgatum* L.) and tall fescue (*Festuca arundinacea* Schreb.), were produced from 2007 to 2011 in France, Belgium, Germany and Luxembourg. For spelt, grains were separated from straw at the harvest. Rye was harvested as an immature cereal for silage production. Some maize and sorghum were harvested at the end of the winter in March, in order to produce biomass with low moisture content. After the harvest, each sample was packed in plastic bags and sealed under vacuum to allow a silaging process. Fermentation gas due to the silaging process was removed by opening the bag, repacking and resealing the biomass under vacuum. After the end of the silaging fermentation, the sealed bags containing biomass were stored at room temperature until laboratory analysis.

Total solids (TS) content in the wet silages was measured after a drying step in an oven at 105°C for 24h, and volatile solids (VS) content in the wet silages was quantified subsequently after combustion in a furnace at 550°C for 6h.

For each wet silage, dry silage powder was produced from a subsample dried for 48h in an oven at 70°C and subsequently ground in a centrifugal mill (Cyclotec, Foss) through a 1-mm mesh.

2.2. BMP measurements

The method to measure the BMP of the produced biomass was described in details in previous papers by Mayer et al. (2014a; 2014b), following the recommendations of the VDI 4630 standard (Verein Deutscher Ingenieure, 2006). In brief, batch assays were carried out in 2L bottles. The digesters were filled with an inoculum collected in an anaerobic bioreactor of a wastewater treatment plant (SIVEC, Schifflange) and with the wet sample of energy crop silage to analyse. The produced biogas was collected in gas bags through tubing. The tubes were cooled to condense water vapour, except for batch assays involving maize samples. The collected biogas was manually (for maize) or automatically (for other energy crops) analysed for its volume and composition (CH_4 and CO_2), with a TG05 wet drum-type gasmeter (Ritter, Germany) and specific infrared sensors (Dynament, UK) respectively. Gas volumes were normalised (273 K, 1013 hPa) according to the measured temperature and pressure conditions. The endogenous gas production of the inoculum was subtracted for each gas measurement. BMP measurements were carried out in triplicates only for maize samples. A single batch assay per sample was realized for other samples. BMP assays were run simultaneously with microcrystalline cellulose as a standard to assess the inoculum activity.

At the end of the batch assays, biomethane productions were cumulated to obtain the BMP. The BMP were expressed on crude matter basis (BMP_{CM}) and then expressed on a volatile solids basis (BMP_{VS}) with the VS content of the wet ensiled biomass.

2.3. NIRS measurements

Before carrying out the BMP batch assays, NIR spectra of both wet silages (wet NIRS) and dry silage powders (dry NIRS) were measured, as described in Chapter 4. In brief, a sampling cup was filled with the wet biomass or the dry and ground biomass to measure the NIR reflectance spectrum of the sample with a Bruker MPA spectrometer (Bruker Optik GmbH) operated with OPUS 6.5. This procedure was realized three times per sample to obtain triplicate measurements. Absorbance spectra were collected in the 3594-9989 cm^{-1} range with a resolution of 16 cm^{-1}. The measured NIR spectrum of a sample was the result of the average of 32 spectra automatically collected during the rotation of the sample cup.

2.4. Data treatment and chemometrics

The number of samples, the mean, the standard deviation (SD) and the standard error of laboratory (SEL) were calculated with Excel 2010 (Microsoft Corp.).

Model calculations were carried out with Matlab, version R2012a (MathWorks) and PLS_Toolbox, version 7.3.1 (Eigenvector Research Inc.).

Before any model calculation, the maximum coefficient of determination of any calibration (R^2_{max}) was calculated according to Dardenne (2010) using:

$$R^2_{max} = \frac{SD^2 - SEL^2}{SD^2}$$

For the modelling procedure, the triplicate NIR spectra of a sample were averaged to obtain one single spectrum per sample. Triplicate and average spectra were plotted to check outliers before being used in the model calculation as predictors. The regression method used was the partial least squares (PLS) for all models.

Preprocessing methods included mean centering (MC), autoscaling (AS), standard normal variate (SNV) or detrend (Det). Derivatives were also assessed and abbreviated as xD(y;z), with x the derivative order, y the polynomial order and z the filter width. The excluded spectral data were used in the derivative calculations, but not in the subsequent model.

A cross-validation was used during the calibration procedure to find the optimal number of latent variables (LV) for multivariate models. The cross-validation consisted in the random split of the calibration set in 10 segments, with the same number of samples in each segment. The calibration model was then calculated with all the samples from 9 segments. Using this calibration model, the value of the parameter was predicted for each sample of the 10th segment. This procedure was repeated 10 times to calculate the statistics of the model.

The root mean square error (RMSE), the coefficient of determination (R^2) and the ratio of standard error of prediciton to sample standard deviation (RPD) was calculated for the calibration (RMSEC, R^2C, RPDC) and cross-validation (RMSECV, R^2CV, RPDCV).

The RMSE was calculated according to:

$$RMSE = \sqrt{\frac{\sum(y_m - y_p)^2}{N}}$$

with y_m the measured values, y_p the predicted values and N the number of samples in the dataset.

The R^2 was calculated according to:

$$R^2 = \left(\frac{\sum(\widehat{y_m} - \overline{y_m})^2}{\sum(y_m - \overline{y_m})^2}\right)$$

With y_m the measured values, $\widehat{y_m}$ the estimated y_m values given by the regression line and $\overline{y_m}$ the mean y_m value.

The RPD, which is a more discriminant value than the R^2 value, was calculated according to:

$$RPD = \frac{1}{\sqrt{1 - R^2}}$$

The intercept and the slope were calculated for the linear regression between the predicted values (x-axis) and the measured values (y-axis) for the cross-validation data. The best model was chosen and presented in the present paper according to the lowest RMSECV, the lowest number of LV and the highest R^2CV and RPDCV.

The variable importance in projection (VIP) scores give a summary of the importance of a predictor for both predictors and predicted parameter. A variable with a VIP score close to or greater than 1 can be considered as important in a given model (Wold et al., 2001). The VIP score for the j-th variable was calculated according to:

$$VIP_j = \sqrt{p \frac{\sum_{k=1}^{h}\left(b_k^2 t_k^t t_k \left(\frac{w_{jk}}{\|w_k\|}\right)^2\right)}{\sum_{k=1}^{h} b_k^2 t_k^t t_k}}$$

with p the number of predictors, h the number of LV, k the k-th LV, b the regression coefficient of the score matrix, t the column vector of the score matrix, w the column vector of the weight matrix (Chong & Jun, 2005).

The SEL of prediction of each parameter for the calibration set was calculated by applying the model to spectra replicates in order to obtain the predicted values.

3. Results and discussion

3.1. Prediction of the VS from NIRS

The models that predict the VS content of energy crop silages from wet NIRS or dry NIRS are presented in Table 8.1 and Figure 8.1. The wet NIRS model has a high RPDCV of 6.5 and a low RMSECV of 2.7 %CM. The slope (1.00) and the intercept (0.01) of the linear regression between the predicted and the measured VS contents showed an ideal prediction along the range (Figure 8.1A). The VS content of 1069 silage samples from 12 plant species was very well predicted with the wet NIRS model. The prediction of the VS content of a single specie (371 maize silages) had a RMSECV of 1.5%CM (Chapter 4, Table 4.3). The prediction of the VS content of biomass was more accurate when using models corresponding to a specific plant species. Such models should be developed if samples of a specific plant species are numerous and present variability of the VS content.

Figure 8.2 shows the VIP scores of the wet NIRS and dry NIRS models that predict the VS content of wet silages of various energy crops. Two peaks were identified at 5369 and 5276 cm^{-1} and correspond to the water absorbance region (Conzen, 2006). The moisture content of these plant species is used to determine the VS content. The low ash content of all these plant species (less than 6 %CM, Chapter 7) could explain the ability of the NIRS model to predict the VS content through the moisture content. The similar explanation was given for the prediction of the VS content of wet maize silages specifically, with NIR spectra of the wet biomass (Chapter 4).

The model that predicts the VS content from dry NIRS showed a lower RPDCV of 2.1 as compared to the wet NIRS model (Table 8.1). The moisture was removed in dry energy crops silages. The spectral information about the moisture content of wet energy crops silages was thus not directly available in the dry NIRS model. The dry NIRS model could make use of correlation between the biochemical composition of the dry energy crop silage powders and the moisture content of the wet silages, as shown for maize silages in Chapter 4. However, the dry NIRS model was not appropriate to accurately predict the VS content of energy crops.

Table 8.1. Prediction models of the volatile solids (VS) content and the biochemical methane potential on a crude matter basis (BMP_{CM}) of wet energy crops with near infrared spectroscopy (NIRS).

Calibration and cross-validation				
Predictor	Wet NIRS		Dry NIRS	
Parameter	VS	BMP_{CM}	VS	BMP_{CM}
Unit	%CM	$mL.gCM^{-1}$	%CM	$mL.gCM^{-1}$
N	1069	884	996	809
Outliers	0	0	0	0
Min	10.7	39	11.5	39
Mean	32.0	120	32.2	120
Max	88.8	318	88.8	318
SD	17.2	46.5	16.7	44.5
SEL	0.90	5	0.90	5
R^2_{max}	1.00	0.99	1.00	0.99
Range	9002-3903	9002-3903	9002-3903	9002-3903
Step	8	8	8	8
Predictor preprocess	2D(3;15); MC	2D(3;15); MC	SNV; Det-2D(3;15); MC	SNV; Det-2D(3;15); MC
Reg. method	PLS	PLS	PLS	PLS
Cross-validation	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)	Rdm(10;10)
LV	4	5	4	7
RMSEC	2.7	14	7.7	22
R^2C	0.98	0.92	0.79	0.75
RPDC	6.5	3.5	2.2	2.0
RMSECV	2.7	14	7.9	23
R^2CV	0.98	0.92	0.78	0.73
RPDCV	6.5	3.5	2.1	1.9
Intercept	0.01	0.18	0.12	1.42
Slope	1.00	1.00	1.00	0.99
SEL prediction	0.98	4	0.76	5

Wet NIRS is NIRS measured on wet maize silages and dry NIRS is NIRS measured on dry maize silage powders. The SEL was calculated by Mayer et al. (2014a). 2D: 2nd derivative, CM: crude matter, Det: detrend, LV: latent variables, MC: mean-center, N: sample number, PLS: partial least squares, $R^2C/CV/P$: coefficient of determination of calibration/cross-validation/validation, RMSEC/CV/P: root mean square error of calibration/cross-validation/validation, RPDC/CV/P: ratio of standard error of prediction to sample standard deviation for calibration/cross-validation/prediction, SEL: standard error of laboratory, SEP: standard error of prediction, SNV: standard normal variate, VS: volatile solids.

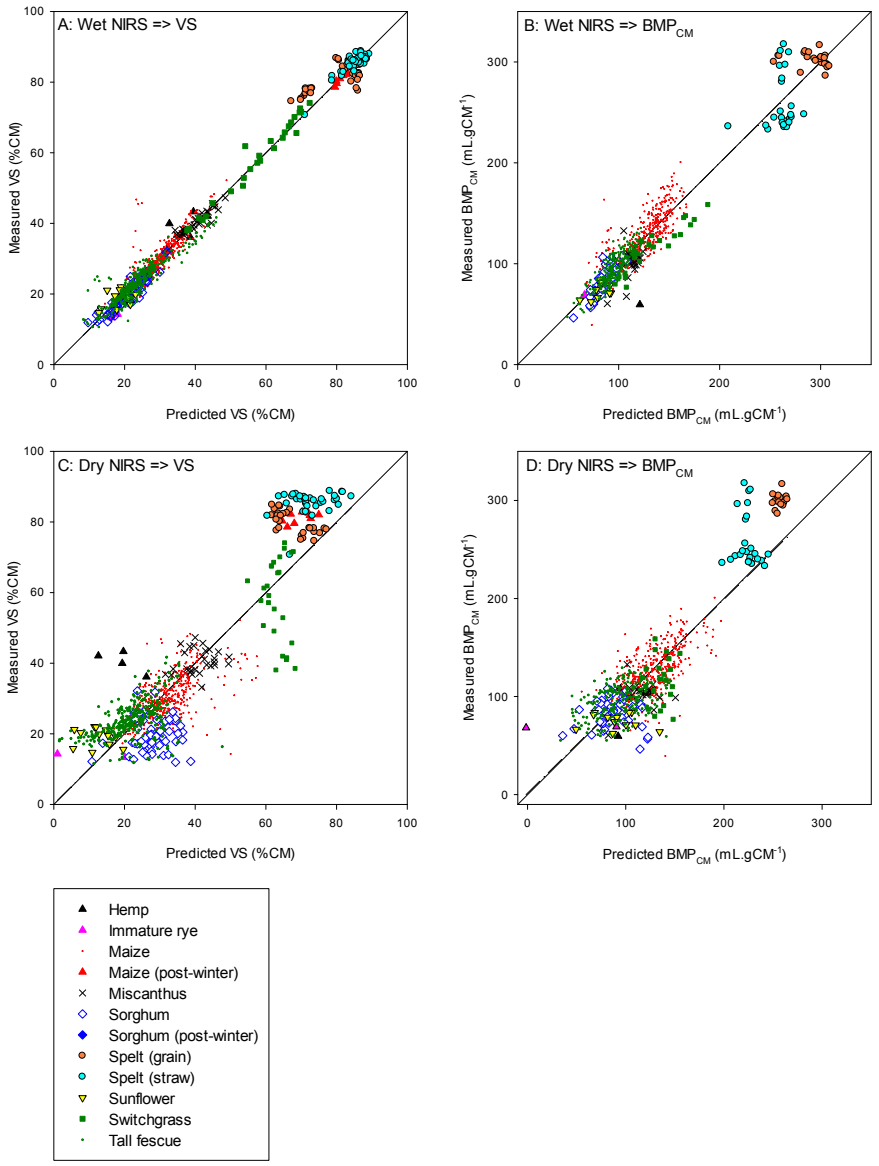

Figure 8.1. Measured and predicted values with near infrared spectroscopy (NIRS) of the volatile solids (VS) contents (A and C) and the biochemical methane potentials on a crude matter basis (BMP_{CM}, B and D) of wet energy crops silages. Wet NIRS (A and B) is NIRS measured on wet energy crops silages and dry NIRS (C and D) is NIRS measured on dry energy crops silage powders.

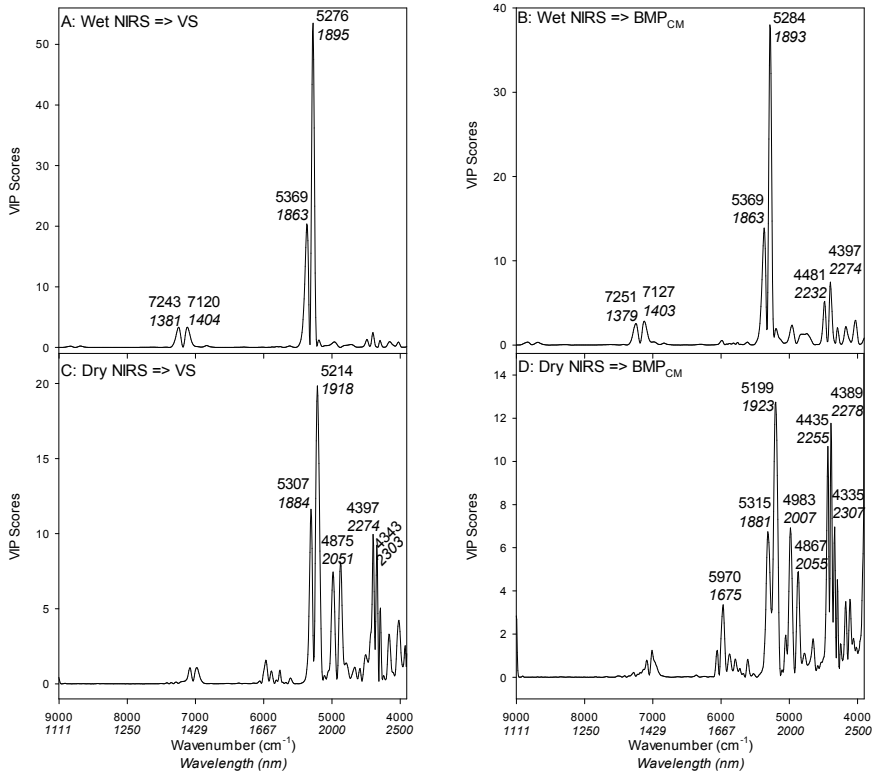

Figure 8.2. Variable Importance in Projection (VIP) scores of models that predict the volatile solids (VS) content (A and C) and the biochemical methane potential (BMP) on a crude matter basis (BMP_{CM}) of wet energy crop silages (B and D) with near infrared spectroscopy (NIRS). Wet NIRS (A and B) is NIRS measured on wet energy crops silages and dry NIRS (C and D) is NIRS measured on dry energy crops silage powders.

3.2. Prediction of the BMP_{CM} from NIRS

The prediction of the BMP_{CM} of wet energy crop silages with NIRS model is presented in Table 8.1 and Figure 8.1. The RPDCV of 3.5 indicated that the model was good for screening. The error of prediction (RMSECV of 14 $mL.gCM^{-1}$) was less accurate than the repeatability of the BMP assay (SEL of 5 $mL.gCM^{-1}$). Wet NIRS model can be used to obtain quickly a measurement of the BMP_{CM} of various energy crop silages. However, the BMP assay can't be avoided if accurate measurements are needed.

The prediction of the BMP_{CM} of wet maize silages only with a wet NIRS model had a RMSECV of 10 $mL.gCM^{-1}$ (Chapter 5, Table 5.4). Similarly to the prediction of the VS content of wet energy crop silages, the prediction of the BMP_{CM} of biomass was more accurate when a model was calibrated for a specific plant species. If the sample number is sufficient for a plant species, models corresponding to a single plant species should be developed to increase the accuracy of the prediction of the BMP_{CM} of energy crops silages. Only maize was thus explored in the present work, but a model for tall fescue could be also investigated with the present data.

Predicted BMP_{CM} values for switchgrass, miscanthus and hemp were higher on average than the measured BMP_{CM} values (Figure 8.1B). This bias can be explained by the incomplete digestion of the samples that continued to produce biomethane at the end of the BMP assays, as concluded by Mayer et al. (2014b).

The VIP scores of the models that predict the BMP of wet energy crop silages with NIRS are presented in Figure 8.2. For wet NIRS models, the VS content and the BMP_{CM} have the same important spectral predictors, as shown by two major peaks around 5369 and 5276-5284 cm^{-1} (Figure 8.2 A and B). For wet maize silages, similar high VIP scores, characterizing moisture absorbance, were also observed in a similar spectral range. Moreover, the BMP_{CM} of wet silage crops was shown to be influenced mainly by the VS content (Chapter 7, Figure 7.4). Similarly to the prediction of the BMP_{CM} of wet maize silages, wet NIRS model made use of the spectral information about the moisture content in the biomass on one hand, and the correlation between the moisture content, the VS content and the BMP_{CM} on the other hand, to predict the BMP_{CM} of wet energy crop silages.

3.3. Prediction of the BMP_{VS} from NIRS or from the plant species

The prediction of the BMP_{VS} of wet energy crop silages from wet NIRS and dry NIRS is presented in Table 8.2 and Figure 8.3.

The R^2_{max} value for the prediction of BMP_{VS} of 12 different ensiled energy crops was 0.89 and higher than the R^2_{max} for the model including only wet maize silages (R^2_{max} = 0.72, Table 5.1). The variability for the set including various plant species (SD of 68.2 $mL.gVS^{-1}$) was higher than for the set including maize only (SD of 41.4 $mL.gVS^{-1}$). The R^2_{max} was improved by such larger range of values and not by the accuracy of the measurement.

NIRS models that predict the BMP_{VS} of various biomasses have high RMSECV (44 and 51 $mL.gVS^{-1}$ for wet NIRS and for dry NIRS, respectively, Table 8.2). The RMSECV was lower for NIRS models that predict the BMP_{VS} of only wet maize silages (34 $mL.gVS^{-1}$, Table 5.4). Similarly to the prediction of the BMP_{VS} of wet maize silages (Chapter 5), the prediction of the BMP_{VS} of various biomasses was not accurate. Moreover, a model specific to plant species such as the one for maize, improved the accuracy.

According to Figure 8.1A, the wet NIRS model discriminitated the samples with high fibre content such as miscanthus, switchgrass and spelt from the other samples. The plant species was thus investigated as a predictor of the BMP_{VS} of wet energy crop silages. More precisely, the mean BMP_{VS} was calculated for each plant species (Chapter 7, Figure 7.1) and used as the predicted BMP_{VS} of a sample. The RMSEC was 47 $mL.gVS^{-1}$ with such a model, which was comparable to the RMSEC of wet NIRS and dry NIRS models (43 and 49 $mL.gVS^{-1}$ respectively, Table 8.2). Such values correspond to around 11-12% of the mean BMP_{VS}. A similar accuracy was thus reached to predict the BMP_{VS} when using spectral data or the plant species of the sample as predictor. The fastest and more accurate predictor of the BMP_{VS} of wet energy crops assessed in this study was the average BMP_{VS} of the plant species to which the sample belonged.

Table 8.2. Prediction models of the biochemical methane potential on a volatile solids content basis (BMP_{VS}) of wet energy crops with near infrared spectroscopy (NIRS).

Calibration and cross-validation		
Substrate	Wet energy crops	
Parameter	BMP_{VS}	
Unit	$mL.gVS^{-1}$	
Predictor	Wet NIRS	Dry NIRS
N	884	808
Outliers	0	0
Min	124	124
Mean	399	397
Max	699	699
SD	68.2	67.8
SEL[a]	22	22
R^2_{max}	0.89	0.89
Range (cm^{-1})	9002-3903	9002-3903
Step (cm^{-1})	8	8
Predictor preprocess	2D(3;15)-MC	SNV-Det-2D(3;15)-MC
Reg. method	PLS	PLS
Cross-validation	Rndm(10;10)	Rndm(10;10)
LV	7	8
RMSEC	43	49
R^2C	0.61	0.47
RPDC	1.6	1.4
RMSECV	44	51
R^2CV	0.59	0.43
RPDCV	1.6	1.3
Intercept	7.0	17.5
Slope	0.98	0.96
SEL prediction	10	6

Wet NIRS is NIRS measured on wet maize silages and dry NIRS is NIRS measured on dry maize silage powders. The SEL was calculated by Mayer et al. (2014). 2D: 2nd derivative, Det: detrend, LV: latent variables, MC: mean-center, N: sample number, PLS: partial least squares, R^2C/CV/P: coefficient of determination of calibration/cross-validation/validation, RMSEC/CV/P: root mean square error of calibration/cross-validation/validation, RPDC/CV/P: ratio of standard error of prediction to sample standard deviation for calibration/cross-validation/prediction, SEL: standard error of laboratory, SEP: standard error of prediction, SNV: standard normal variate, VS: volatile solids.

The variability of the BMP_{VS} of energy crops appears as a difficult parameter to predict. For wet maize silages, this was explained by a SEL of the BMP bach assay that was too large as compared to the SD of the measured BMP_{VS} (Chapter 5). The use of dry and ground silage powder was proposed to improve the SEL of the BMP assay. It showed succesfull results for maize silages (Figure 6.1, Chapter 6) and should be investigate on various plant species.

Prediction of the BMP_{VS} of various organic substrates with NIRS was reported to have RMSECV of 31 $mL.gVS^{-1}$ for 74 samples (Lesteur et al., 2011) and 42 $mL.gVS^{-1}$ with 171 samples after removing 72 outliers (Doublet et al., 2013). Similar results were obtained in the present study for wet energy crops. The authors also pointed out the level of error of the BMP assay as a source of uncertainty of the BMP_{VS} prediction. The improvement of the repeatability of the BMP assay appears as a necessary step to increase the accuracy of the BMP_{VS} prediction for all substrates.

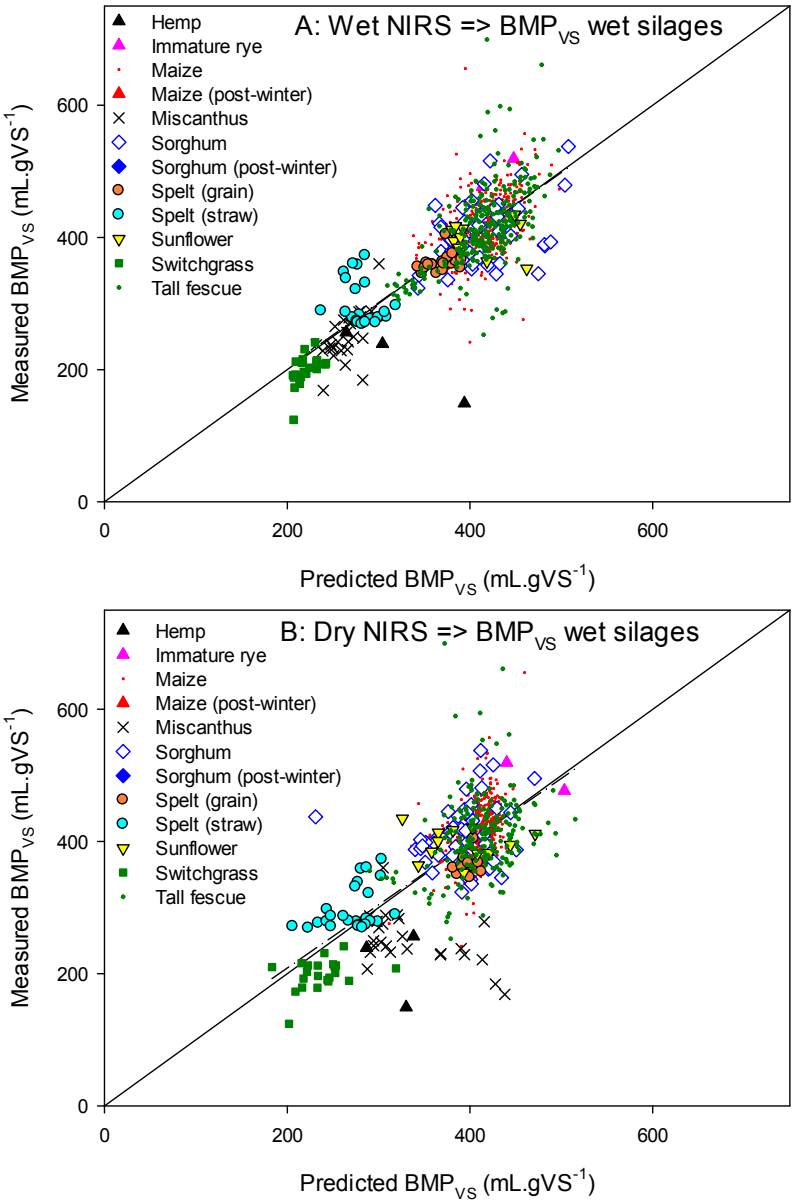

Figure 8.3. Measured and predicted biochemical methane potential on a volatile solids content basis (BMP_{VS}) of wet energy crop silages with near infrared spectroscopy (NIRS). Wet NIRS (A) is NIRS measured on wet maize silages and dry NIRS (B) is NIRS measured on dry maize silage powders.

4. Conclusion

Models based on NIRS can predict the VS content and the BMP_{CM} of wet energy crops silages with RMSECV of 2.7 %VS and of 14 $mL.gCM^{-1}$, respectively, representing 8% and 12% of the respective means.

Prediction of the BMP on a VS basis was less performing according to the RPDCV of 1.6, because of the narrow range of BMP_{VS} observed for the various crops tested, as compared to the accuracy of the BMP assay. The BMP_{VS} of wet energy crops silages was predictable with a RMSECV of 44 $mL.gVS^{-1}$ with wet NIRS, corresponding to 11% of the mean BMP_{VS}. Knowing the plant species was sufficient to predict the BMP_{VS} with a RMSEC of 47 $mL.gVS^{-1}$, corresponding to 12% of the mean BMP_{VS}.

As previoulsy shown for wet maize silages (Chapter 5), models specific to each plant species can potentially improve the prediction. Improvement of the repeatability of the BMP assay must be considered to increase the accuracy of the prediction results.

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Highlights of Chapter 8

- The VS content and the BMP_{CM} of crude wet energy crops can be predicted with models based on NIRS.
- The BMP_{VS} of crude wet energy crops is poorly predicted, regardless the predictor.
- Models specific to plant species can potentially improve the predictions.

Chapter 9

Discussion, conclusion and perspectives

Biomethanation is a sustainable way to produce biomethane as a source of renewable energy. The primary resource to produce biomethane with such a process consists in biomass. Among all biomasses, energy crops are particularly used for anaerobic digestion and the highest biomethane yield per unit of cropped area is looked for maximizing the biomethane production. Such investigations need the measurement of the BMP with a batch assay that is time-consuming.

In this context, the aim of the PhD thesis is to answer to two main questions:

- **What are the characteristics of energy crops with a high biomethane yield?**
- **Can the biochemical composition or near infrared spectroscopy be used in models that quickly predict the BMP of energy crops?**

These two questions are discussed and answered thereafter based on the results obtained in the previous chapters, taking into account the framework of the study and its limitations.

Framework of the studie and limitations

The sample set of energy crops studied in the present work is characterised by specific plant species and varieties, harvested at various maturities in different cropping environments. The 1147 different samples are representative of plant species usually cropped in the Greater Region, except some maize, sorghum and miscanthus samples harvested in the winter (Chapter 7). In the context of anaerobic digestion, this work gives a broad view of the performance of some representative energy crops, without highlighting precisely the optimal conditions (such as the maturity at the harvest) to maximize biomethane production. Investigation of the influence of cropping conditions was restricted to maize samples (Chapter 3). Such investigations remain to be performed with the other plant species and under various environmental conditions.

Similarly, the prediction models based on the biochemical composition or NIRS were developed for the samples included in the present work. Their application to other biomasses would require further validation.

What are the characteristics of energy crops with a high biomethane yield?

Energy crops can be used in anaerobic digestion to produce biomethane. The highest biomethane yield per hectare of cropped area is thus looked for maximizing the enegy production. The biomethane yield is the result of the product between the biomass yield and the BMP. Both parameters can thus affect the biomethane yield of energy crops (Chapter 7), as well as parameters such as the cropping environment, the variety or the harvest date studied more specifically for maize silages (Chapter 3).

Maize (annual plant) and miscanthus (perennial plant) have the highest biomethane yields among the tested energy crops (6934 and 4468 $m^3.ha^{-1}$ on average, Figure 7.1). More precisely, these plant species have similar biomethane yields when cropped in similar conditions (5508 and 5322 $m^3.ha^{-1}$, Figure 7.2). For maize specifically, the cropping environment has the main effect on the biomethane yield (Figure 3.1), whereas the variety and the harvest date do not have a significant effect on the biomethane yield (Figure 3.1 and Figure 3.2).

On one hand, the biomethane yield of energy crops is mainly influenced by the $biomass_{VS}$ yield (R^2 = 0.94, Table 7.4 and Figure 7.5). In particular for maize silages, the $biomass_{VS}$ yield has a higher effect on the biomethane yield (R^2 = 0.83, Figure 3.4) than the BMP (r = 0.65, Table 3.3). Late maturing maize varieties tend to produce higher biomass yields than early varieties (Figure 3.1 and 3.2).

On the other hand, the BMP is influenced by the plant species and their fibre contents (Figure 7.4). More specifically, plant species with high fibre content such as miscanthus are more slowly digested than maize (Figure 7.3). For crude wet maize silages, the BMP_{VS} slightly decreases with the VS content whereas it is more constant for dry maize silage powders (Figure 3.3 and Figure 6.2).

In conclusion, the ideal energy crop for biomethanation is thus characterized by the production of a high biomass yield in the field and a biochemical composition that does not limit its anaerobic digestibility. The recalcitrant fibre content should be limited. In this context, maize is the best annual energy crops for biomethanation. Late varieties producing high biomass yields harvested at an early stage characterized by a low VS content are advised for biomethane production.

Miscanthus is a perennial plant that can compete with maize due to its high biomass yield, but it is digested more slowly.

Improvement of the biomass yield

The $biomass_{VS}$ yield per hectare is the most significant parameter to influence the biomethane yield per hectare (R^2 = 0.94, Table 7.4). The increase of the amount of produced biomass, while maintaining their quality in terms of anaerobic digestibility, is thus a way to improve the biomethane yield of energy crops. Such an increase can be realized through breeding strategies and the selection of high yielding varieties, for instance late varieties for maize, or through cropping practices such as the choice of optimal harvest date, for instance early harvest for maize (Chapter 3).

Improvement of the BMP

In order to maximize the biomethane yield of energy crops, efforts orientated towards biomass quantity production are thus considerd to be more promising than improving their anaerobic digestibility, that probably results from long lasting efforts deployed to improve forage quality through breeding. However, the anaerobic digestibility of plant species can be improved as shown by the highest BMP of miscnathus harvested before the winter as compared to the low BMP of miscanthus usually harvested after the winter (higher than 200 $mL.gVS^{-1}$ and 84 $mL.gVS^{-1}$ respectively, Chapter 7). The effect on the BMP of the maturity stage at the harvest should be investigated more specifically for all energy crops, similarly to maize.

The digestion kinetic must also be considered with the anaerobic digestibility of energy crops. Miscanthus silages harvested before the winter have a higher BMP calculated at an infinite time than maize silage, while being digested more slowly because of the recalcitrant fibre fractions (Figure 7.3). The biomethane yield of miscanthus would then be higher with BMP measured after longer batch assays. Such long assays should be investigated to highlight the maximum gas production of fibrous plant species. Moreover, the effect on the kinetic digestion of the plant

species, the variety or date of harvest, as well as the biochemical composition, should also be investigated to better understand the biomethane production.

In addition to agronomic approaches, other technical ways exist to improve the BMP. Pretreatments, including thermo-chemical degradation or biological hydrolysis can improve the kinetic of the anaerobic digestibility. However, such pretreatments are costly and should not negatively affect the environmental and economic balance of biomethane production through anaerobic digestion of energy crops.

Long retention time in anaerobic reactors is another alternative to allow the full expression of the BMP. Agricultural biogas plants typically have long digestion time and offer thus perfect condition to plant species that are slowly degraded and converted in biomethane.

Perspectives for energy crops used in biomethanation

For maize specifically, the cropping environment has the largest effect on the biomethane yield (Chapter 3). In the context of the development of energy crops in Greater Region, agronomic trials should be conducted in various locations, characterized by different soils and climates, to select the best agronomic practices for energy crops dedicated to biomethanation. Marginal lands should even be investigated for energy crops to save fertile areas dedicated to feed and food production.

For perennial crops, the cropping of green miscanthus harvested before the winter is an alternative to annual maize because of its high biomass yield (Figure 7.2, Chapter 7). Miscanthus cropping should be assessed on a long term over its lifespan (10-15 years) to conclude on its potential for biomethane production. A trial, longer than the one conducted in Chapter 7, comparing perennial miscanthus harvested in a period between autumn and winter, and annual maize harvested in autumn, should be investigated with an emphasis on the maturity stage at the harvest, in order to assess the biomethane production over years, but also the economic and environmental impact of both crops.

A study in a large-scale pilot should confirm the potential of the tested energy crops and their influence on the digestate. Finally green chemistry or other biofuels could take advantage of the present research about energy crops to increase their yields.

Major highlights about energy crops for biomethanation

- The biomethane yield of crude wet silages of energy crops is mainly influenced by their $biomass_{VS}$ yield.
- Miscanthus harvested before the winter and maize produce the highest biomethane yields in the Greater Region.
- Plant species with high fibre content such as miscanthus are slowly digested.

For maize silages specifically:

- The cropping environment is responsible for most of the variability of the biomethane yield.
- Late maturing maize varieties harvested at an early stage should be investigated to reach high biomethane yields.

Can the biochemical composition or near infrared spectroscopy be used in models that quickly predict the BMP of energy crops?

The BMP of energy crops allows to determine their biomethane yields. It is measured with a time-consuming method that involves many scientific instruments. Faster methods were investigated in the present work.

Prediction of the BMP_{CM} with biochemical informations

The biomethane is produced through the conversion and degradation of organic molecules of the biomass. The biochemical composition of energy crops was used to predict the BMP_{CM} in a first step.

For the tested samples, the anaerobic digestibility of most plant species is similar (on average, BMP_{VS} of 395 ± 2 $mL.gVS^{-1}$, as determined by linear regression, Figure 7.4), except for fibrous plant species such as miscanthus, hemp or switchgrass, which have lower measured BMP_{VS}. Such low variability allows high correlations between the VS content and the BMP_{CM} of energy crops and especially maize (R^2 of 0.80 and 0.81 respectively, Table 7.3 and Figure 3.3). Thus, the VS content of silages can be used to predict the BMP_{CM} of energy crops and of maize silages in particular, with SEE of 21 and 11 $mL.gCM^{-1}$ respectively (Table 7.3 and Figure 3.3).

For wet maize silages specifically, informations about the biomass composition in addition to the VS content do not highly increase the accuracy of the prediction of the BMP_{CM} (RMSEC of 10 $mL.gCM^{-1}$, Table 5.2). However, the biomass composition allows to predict the BMP_{CM} of dry maize silage powders with a RMSECV of 5 $mL.gCM^{-1}$ (Table 6.1).

These prediction errors are considered as low for maize silages as compared to the repeatability of the BMP batch assay (SEL of 5 $mL.gCM^{-1}$, Table 5.1 and Table 6.1) and to the mean BMP_{CM} of wet maize silages, dry maize silage powders and their standard deviations (126 ± 25 and 315 ± 15 $mL.gCM^{-1}$ respectively; Table 3.2 and Table 6.1). The prediction error being higher for wet energy crops, a prediction model for each plant species should thus be developed to potentially improve the prediction accuracy, as seen for maize.

Prediction of the BMP_{CM} with NIRS

Models based on wet NIRS successfully predict the VS content of crude wet silages of energy crops and maize especially with RMSECV of 2.7 and 1.5 %CM (Table 8.1 and Table 4.3). Thus, thanks to the correlation between the VS content and the BMP_{CM} presented above, models based on wet NIRS can predict the BMP_{CM} of crude wet silages of energy crops and maize especially with RMSECV of 14 and 10 $mL.gCM^{-1}$ (Table 8.1 and Table 5.4). Models based on wet NIRS are thus accurate to predict the BMP_{CM} of crude silages of energy crops and maize specifically. Moreover, the prediction of the BMP_{CM} of biomass is faster with NIRS (few minutes) rather than measuring the VS content (at least one day).

For dry maize silage powders, models based on dry NIRS can predict the BMP_{CM} with a RMSECV of 7 $mL.gCM^{-1}$ (Table 6.1). This model is slightly less accurate than the model using the biochemical composition presented above. However, the prediction using NIRS is faster than the analysis of the biochemical composition.

In conclusion, the BMP_{CM} of the substrate digested in the BMP assay can be accurately predicted with the NIR spectra of this substrate. This is of particular importance for on-line implementation and direct measurement.

Prediction of the BMP_{VS}

Model based on wet NIRS predict the BMP_{VS} of crude wet silages of energy crops or maize with RMSECV of 44 or 34 $mL.gVS^{-1}$ respectively (Table 8.2 and Table 5.4). A model using the biochemical composition of maize silages predict their BMP_{VS} with a RMSECV of 36 $mL.gVS^{-1}$ (Table 5.4). Moreover, knowing the plant species is sufficient to have an estimation of the BMP_{VS} of crude wet silages of energy crops with a RMSEC of 47 $mL.gVS^{-1}$ (Chapter 8). Thus, considering the mean BMP_{VS} of crude wet silages of energy crops or of maize and their standard deviations (399 ± 68 and 418 ± 41 $mL.gVS^{-1}$; Table 8.2 and Table 5.4), the BMP_{VS} of wet energy crops or wet maize silages are poorly predicted regardless the predictor, as compared to the BMP_{CM}. This is probably due to a low repeatability of the BMP assay (SEL of 22 $mL.gVS^{-1}$ calculated for wet maize silages, Table 5.1) within a relatively narrow measured BMP_{VS} range (SD of 68 and 41 $mL.gVS^{-1}$ for energy crops and wet maize silages, Table 8.2 and Table 5.1). Indeed, the measured

anaerobic digestibility of most of the presented energy crops is not highly variable, except when comparing species with high or low fibre contents. Sample sets with higher variability and range of BMP_{VS} and BMP assays with lower uncertainty would be needed to improve the accuracy of prediction.

Perspectives about BMP prediction and NIRS

The VS content mainly influences the BMP_{CM} of wet energy crops silages, and especially of maize silages (R^2 of 0.80 and 0.81 respectively, Chapter 3 and 7). Since the VS content is easily measurable with oven and furnace that are available in most of the laboratories, such correlations will facilitate the measurement of BMP_{CM} of wet energy crops for stakeholders in the biogas sector.

Models based on NIRS determine the biochemical composition of maize silages and the BMP of dry maize silage powders more accurately with spectra measured on dry and ground matter than on wet crude matter (Chapter 4). The removal of moisture and the increased sample homogeneity through grinding improve the information contained in the spectral data. A preprocessing is thus required to improve the accuracy of the prediction, but is time-consuming.

The comparison of VIP scores highlights that models based on wet NIRS use informations about the moisture content to predict the BMP_{CM} of wet energy crops (Chapter 5 and Chapter 8). This is due to the facts (i) that the moisture content gives information about the VS content thanks to a low ash content for the tested biomass (Chapter 7), and (ii) that the VS content and the BMP_{CM} of energy crops are correlated (Chapter 3 and Chapter 7). Thus, VIP scores should be published when models are reported, to potentially understand what parameters are important for the prediction.

Major highlights about BMP prediction of energy crops

- The BMP_{CM} of crude wet silages of energy crops can be predicted with their VS contents and with models based on wet NIRS.
- The BMP_{VS} of crude wet silages of energy crops is poorly predicted regardless the predictor.
- Models specific to plant species can potentially improve the predictions.
- The determination of the R^2_{max} is advised before carrying on modelling procedure.
- The VIP scores of models help to understand what parameters are important for the prediction.

For maize silages specifically:

- The composition of the VS content of wet maize silages do not have a major impact on their BMP_{CM}.
- Biochemical composition and NIRS can be used to accurately predict the BMP of dry maize silage powders.
- The NIR spectra of the substrate digested in the BMP assay can predict the BMP_{CM} of this substrate.
- The BMP_{VS} of dry maize silage powders is lower than the BMP_{VS} of their corresponding crude wet maize silages.

How should the BMP of energy crops be investigated?

The BMP batch assay consists in the anaerobic digestion of a defined amount of substrate in a small bioreactor and the measurement of the produced biomethane over time. In the present PhD work, the anaerobic digestions were stopped after 42 or 56 days for practical reasons. As shown by the gas productions of miscanthus samples (Figure 7.3), the anaerobic digestion can be uncompleted after such a period, especially for biomasses with high fibre content. Curve fitting is then used to calculate the BMP at an infinite time. Comapring the BMP measured at the end of the batch assay and the BMP calculated at an infinite time brings information about the achievement of the batch assay and the digestion kinetic. The digestion kinetic appears then as an important parameter to control when measuring the biomethane production of organic substrate and detailed informations about the degradation rate should also be provided when reporting BMP results.

Some volatile organic compounds such as ethanol, acetic acid or lactic acid, which are present in wet silages, are not included in the measurement of the VS content of energy crops silages. The calculation of the BMP_{VS} of energy crops silages is thus misestimated. A correction should be done on the VS measurement used in the BMP_{VS} calculation, to take into account the loss of volatile organic compounds that are converted in biomethane during the BMP assay.

The BMP batch assay is commonly carried out on the crude wet biomass or on the biomass preprocessed as a dry and ground powder. When performed on the crude wet matter, it allows digesting all the organic matter of the biomass, even the volatile organic compounds such as volatile organic acids and alcohols. Indeed, volatile organic compounds are lost when preparing dry and ground powder and this loss can biase the BMP measurement of around 14 $mL.gCM^{-1}$ (Chapter 6).

On the other hand, the SEL decreases from 15 to 6 $mL.gVS^{-1}$ when using dry maize silage powders instead of wet maize silages (Figure 6.1). The BMP assay carried out on the dried and ground biomass assesses more accurately the anaerobic digestibility of the volatile solids.

Thus, various forms of energy crops should be investigated in BMP batch assays, according to the goal of the study:

- non-ensiled biomass to assess the effect of ensiling on the BMP of various energy crops.
- crude wet substrate to estimate the true biomethane production of the sample, mainly for practical applications.
- wet biomass ground finely (i) to potentially improve the repeatability of the BMP batch assay while preserving all organic compounds and (ii) to assess the effect of the grinding solely.
- dry and ground powder to investigate the effect of the biochemical composition on the BMP of energy crops, with special application in breeding for instance.

To conclude, the characterization of all parameters affecting the BMP assay must be reported in a standardized way, as presented in Table 1.1 and used in Table 3.1 and in Table 7.1, in each study about BMP measurement. Such informations would allow to better compare different studies. Other research works should assess the influence of other parameters such as the scientific material used for the BMP measurement.

Future of NIRS in anaerobic digestion

NIRS is used as a predictor to measure a wide variety of biochemical parameters or properties. Since the monitoring of the anaerobic digestion process is very important to avoid economic losses, NIRS should be developed to be used as a process analytic technology for biomethanation.

For anaerobic digestion, NIRS should be developed to analyse:

- the biochemical composition and the BMP of the feedstock,
- the sludge in the anaerobic digester that can describe the state of the process,
- the composition of the biogas,
- the fertilizing properties (N, P and K) of the residual digestate and its composition.

Three different approaches, with their advantages and inconvenients, should be investigated:

- a laboratory spectrometer equipped with various probes and equipments could analyse many parameters,
- a portable spectrometer could analyse specific parameters for different biogas plants,
- a fixed spectrometer installed on-line and with various channels could analyse specific parameters for an unique biogas plant.

From qualitative measurements to describe the process to quantitative estimation of specific parameters, NIRS can be potentially used as a tool to facilitate the entire management of the anaerobic digestion process.