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On-Site assessment of corn silage biochemical methane potential using a cost-effective NIR device

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ABSTRACT

Corn silage, widely used as a feedstock in anaerobic digestion systems, plays a significant role in renewable energy production. The measurement of the biochemical methane potential (BMP) of ensiled corn at the farm level is of utmost importance for characterizing its methane production potential and optimizing biogas generation. Thus, understanding the BMP of corn silage from individual farms is crucial for efficient biogas plant management and proper utilization of this valuable resource. The study aimed to assess the BMP of corn silage using a cost-effective miniaturized handheld NIR spectrometer with minimal sample preparation. Twenty-nine corn silage wet samples from as many dairy farms located in Lombardy (Northern Italy) were used. NIR calibrations were developed by means of partial least-square (PLS) regression obtaining models with a fairly good coefficient of determination ($R^2_C = 0.92$ and $R^2_{CV} = 0.80$ for DM and $R^2_C = 0.90$ and $R^2_{CV} = 0.75$ for BMP) and a reasonable prediction error ($RMSE_{CV} = 1.38$ and 4.76 for DM and BMP, respectively). Based on these results, the handheld spectrometer would be useful for providing a fast screening of dry matter (DM) and biochemical methane potential (BMP) in corn silage wet samples at farm level, enabling proper management of biogas plants and rapid turnaround in farm advisory systems.

1. Introduction

As it is known, the anaerobic digestion (AD) is a biochemical process wherein biodegradable organic matter undergoes microbial degradation in absence of oxygen, leading to the production of biogas (a methane and carbon dioxide mixture). This process employs a series of complex microbiological processes such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Almeida et al., 2021).

Recently, the companies that take advantage of the anaerobic digestion process have been increasing at a worldwide level. Indeed, the EU biogas produced through AD increased from approximately 8.8 Mtoe in 2010 to 16.6 Mtoe in 2019 with an estimated increment up to 31.5 Mtoe in 2030 (Eurobserv'er, 2020). From a technological point of view, characterizing the substrates used is essential for designing the anaerobic digestion reactor and managing the entire biological process. One of the key parameters to evaluate the total quantity of methane produced by AD, starting from waste or biomass, is the biochemical methane potential (BMP). BMP corresponds to the maximum quantity of methane (m^3 of methane per t of organic matter) potentially produced from a certain substrate in anaerobic conditions (Godin et al., 2015). Conventionally, the BMP is experimentally evaluated in lab-scale by means of a time-consuming (30–90 days or longer, Fitamo et al., 2017; Rodrigues et al., 2019; Almeida et al., 2021) and expensive procedure which is unsuitable in industrial scale. Therefore, to optimize the plant management a fast, reliable, and economical analytical method for BMP determination directly at the AD process plant is needed (Raju et al., 2011; Ward, 2016; Jingura and Kamusoko, 2017).

Modern optical sensing methods have replaced sample characterisation by means of wet-chemical analysis and have successfully been applied in many industry sectors both on-line and at-line. Near-infrared spectroscopy (NIRS) is one of the most versatile sensing methods capable to retrieve qualitative information without sample preparation. Coupled with multivariate techniques (i.e., deep/machine learning methods), NIRS can provide a fast screening of the major components such as water, sugars, dry matter, starch etc. NIR absorbance was found to be related to many organic molecules occur in both fresh and

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processed products (Dull, 1971, 1978, 1986; Murray, 1986; Thuriès et al., 2005; Acharya et al., 2016; Walsh et al., 2020). The food industry has been one of the most studied for the application of these analytical methods. This is due to the strong need to be able to detect any possible non-compliant finished and/or semi-finished product to the production standards in times suitable to the high production rates. NIRS has been implemented in several critical phases for measuring the quality of rice and cereals during growing (Williams and Norris, 1987) or assessing the quality of fruits and vegetables (i.e., dry matter content and acidity, Nicolai et al., 2007). With the same technology, the literature proposes several works based on the estimation of fibres in plant (cellulose, hemicelluloses and lignin) crop residues, and protein, fats and fibre content in animal feed and fodder (González-Martín et al., 2006; Sanderson et al., 1996; Halgerson et al., 2004; Ibáñez and Alomar, 2008; Parrini et al., 2021).

Concerning AD, several process variables have been analysed in anaerobic digesters (Nordberg et al., 2000; Stockl and Oechsner, 2012, Stockl and Lichti, 2018). Almeida et al., 2021 developed a fast and reliable NIR-based model for BMP estimation in a biorefinery context. Doublet et al., 2013 concluded that NIRS appears as a suitable method for fast prediction of the BMP of various organic substrates (i.e., agro-industrial, bio and municipal-solid waste, plants and vegetables, agro-industrial and sewage sludge from wastewater treatment etc.). Fitamo et al., in 2017 successfully applied NIR technology for BMP prediction on various urban organic waste fractions. Triolo et al. (2014) proposed a NIRS model to provide an alternate tool for overcoming the problems of conventional BMP approaches. The authors had moderate success analysing 88 plant biomass samples. Godin et al. (2015) compared models for predicting the BMP of plant biomass, finding NIR models more reliable than those based on chemical composition (lignin, cellulose, hemicelluloses, starch, total soluble sugars, proteins, mineral compounds). In this study, the authors evaluated the fodder BMP of from dried green and silage, and silage-wet. Jacobi et al. (2012) used such technique to predict biogas production from maize silage. Instead, Raju et al., 2011 proposed the latter method to assess the BMP from meadow grasses. NIRS has also been used in full-scale biogas production in a maize silage plant to monitor and predict biogas production (Jacobi et al., 2012, Evangelista et al., 2021). All of these experiences have allowed obtaining good results in BMP prediction but using sophisticated and expensive laboratory instruments.

This study aimed to test a cost-effective, fast, and portable NIR spectrometer for estimating the BMP of corn silage directly at farm level. This instrument could be useful for farmers and agronomists in characterizing corn silage samples taken directly from the silo trench, allowing proper management of the farm biogas plant.

2. Materials and methods

The study was carried out in Lombardy region (Northern Italy) involving 29 dairy farms (within a radius of 50 km, Fig. 1) that produced corn silage in 2022 and stored it in bunker silos.

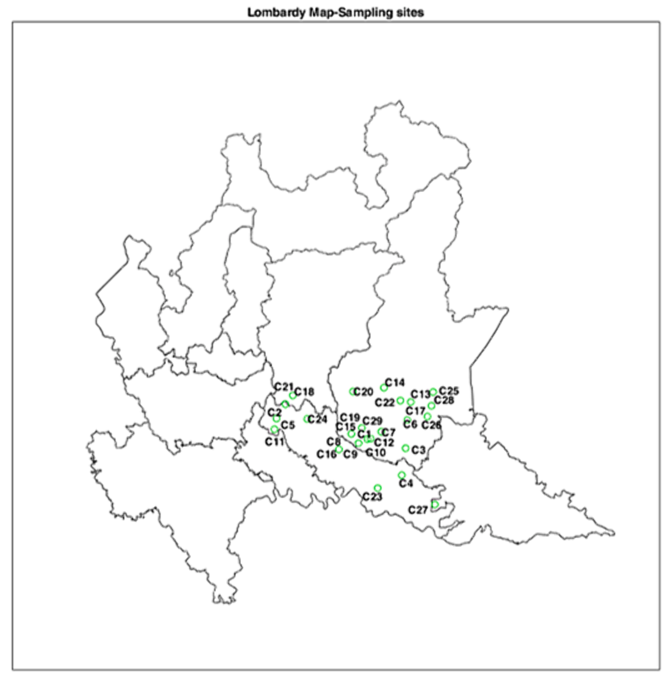

Fig. 1. Lombardy sampling site locations. C2, C4, C5, C8, C11, C16, C23, C24 (Provinces of Cremona); C1, C3, C6, C7, C9, C10, C12, C13, C14, C15, C17, C19, C20, C22, C25, C26, C28, C29 (Provinces of Brescia); C18, C21 (Provinces of Bergamo); C27 (Province of Mantova).

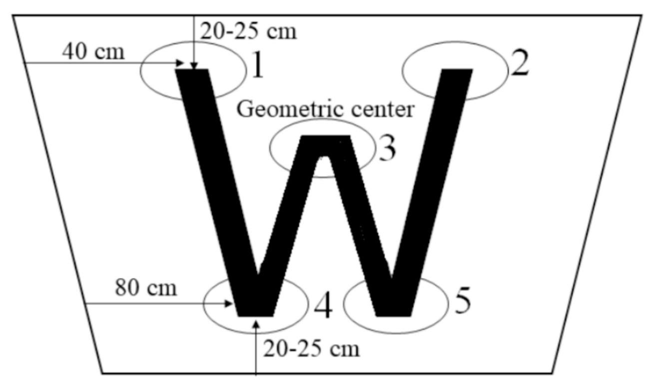

Fig. 2. Silage sampling strategy using the "W" pattern according to Giombelli et al. (2019). Points 1, 2, 4 and 5 are opposite and symmetric; point 3 is in the geometric center of the silo panel. Figure not to scale.

2.1. Sampling

Corn silage samples were taken from side to side and top to bottom following a "W" pattern according to Giombelli et al. (2019). Such sampling method consists of a collection five areas at the ends of a "W" diagram on the silo panel (Fig. 2).

At each silo panel, the five aliquots (approximately 250 g each) were collected using a coring probe combined with a 18 V drill (Stanley Black & Decker, New Britain, Connecticut, USA) and pooled (about 1250 g) to obtain the sample to be analysed. Each pool was then divided into two subsamples for chemical and optical analysis respectively (Fig. 3). This procedure ensured homogeneity and consistent chemical-physical attributes among the two subsamples that were analysed through the application of the latter techniques. Consequently, it facilitated the acquisition of representative samples from each silo panel across every farm, resulting in the collection of a total of 29 samples.

2.2. Chemical analyses

The reference measurements were performed following the ISO 6496:1999 (for the determination of the Dry Matter, DM) and UNI EN ISO 11734:2004 (for the determination of BMP) by an accredited laboratory.

BMP tests involved measuring the maximum amount of methane that can be produced from a given amount of substrate under controlled laboratory conditions. Once drying and grinding, the subsample is mixed thoroughly to ensure homogeneity. The inoculum used in the BMP test is obtained from an anaerobic digester and added to the subsample. The mixture is then transferred to anaerobic reactors, which were incubated at a 38 °C for 4 weeks and daily manually mixed. The biogas (CH_4 and CO_2) volume was estimated by analyzing the reactor

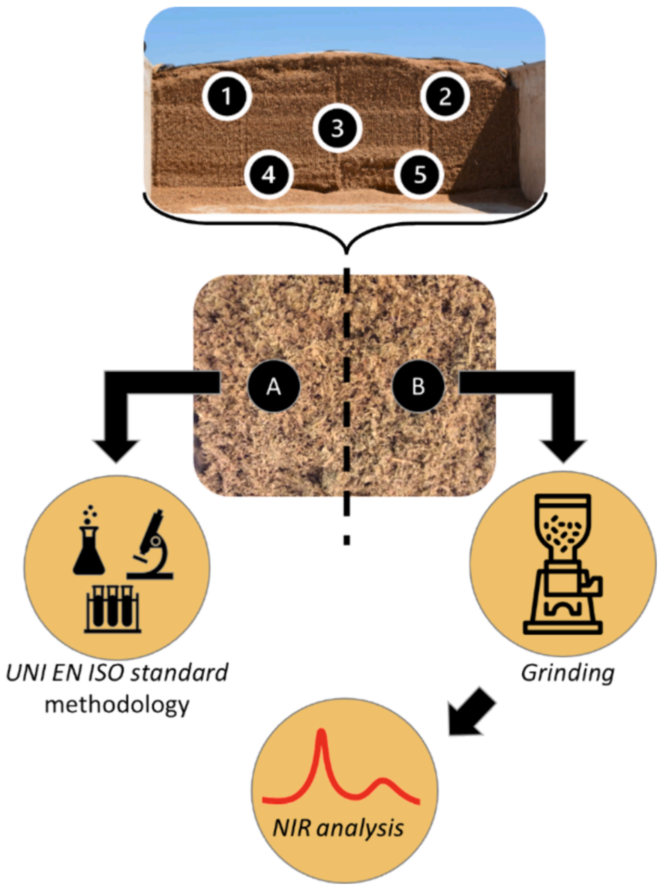

Fig. 3. Sampling procedure description.

headspace pressure using a manometer. The biogas methane concentration was quantified in the lab using an infrared analyzer. BMP production is expressed as m^3 of methane for each ton of substrate (m^3/t).

2.3. Optical analysis

Spectroscopic analyses were performed in lab-scale (simulating the application in real field conditions) after grinding each subsample for 10 s using a household cutter. Possible physical differences (shape and size) between corn subsamples after grinding were taken into account during the data pretreatment in post-processing phase. Therefore, specific data pre-processing techniques were tested in order to drastically reduce any scattering effects caused by heterogeneous subsample sizes.

A handheld NIR device (DLP® NIRscan™ Nano DMD, Texas Instruments, USA) was used to optically scan the grinded silage subsamples from 900 to 1700 nm. The device (Fig. 4) is designed using cost-effective high-performance components combined in a highly customizable small form factor suited for field analysis. The system includes Bluetooth connection and a compact battery to enable hand-held mobile measurements. The optical module employs a pair of tungsten filament lamps positioned in order to avoid any possible specular reflections. Meanwhile, the system collects and concentrates the diffuse reflected light through the slit, before directing the light towards a diffraction grating that disperses it into the various wavelengths. To capture the diffracted radiation, a focusing lens is employed, which then guides it towards a digital mirror array, consisting of an array of micromirrors coordinated to be turned on once illuminated. Finally, the light is conveyed to be measured by a single point InGaAs detector (Texas Instruments, 2017; Rego et al., 2020). Due to the small dimensions (10 mm × 10 mm) of the optical spot for spectral acquisitions it's difficult to collect samples with a particle size larger than the instrument optical. To avoid this drawback and collect as much useful information as possible, each sample (approximately 625 g) was rearranged into a tray (31 x 21 x 4.2 cm) filled to about 3 cm high and levelled. Then, the levelled surface was divided in a 3X4 matrix in order to maximize the area to be optically analyzed (1 sample = 12 replicates). The device was set up to perform 3 scans for each replica and the average spectrum is recorded by the instrument (12 replicates x 29 subsamples = 348 spectra).

2.4. Data processing

Multivariate data analysis was performed in Matlab® environment, version 2022b (The MathWorks, Inc., Natick, MA, USA), using PLSToolbox package (Eigenvector Research, Inc., Manson, Washington) in conjunction with self-made and Matlab® built-in functions.

The Principal Component Analysis (PCA) was applied to explore spectral information and detect any possible outlier both in the NIR optical profiles and in the qualitative analysis based on UNI EN ISO standards (data not shown).

Spectra were measured from 902 nm to 1701 nm. Different spectral transformations (typically used on NIR data) were evaluated (Savitzky-Golay smoothing, Standard Normal Variate, Savitzky-Golay first and second derivative) to remove any possible not significant information that could reduce the predictive performance of the developed regression models. Each pretreatment was tested alone and in combination changing the pre-process settings iteratively in order to optimize the model performance. By the end of the iterative process, the Savitzky and Golay first derivative transformed (Der 1, with a second-degree polynomial order and a window size equal to 9 datapoints) was the most suitable pretreatment able to enhance the resolution, minimizing offsets (baseline vertical shifts) and global intensity effects (commonly

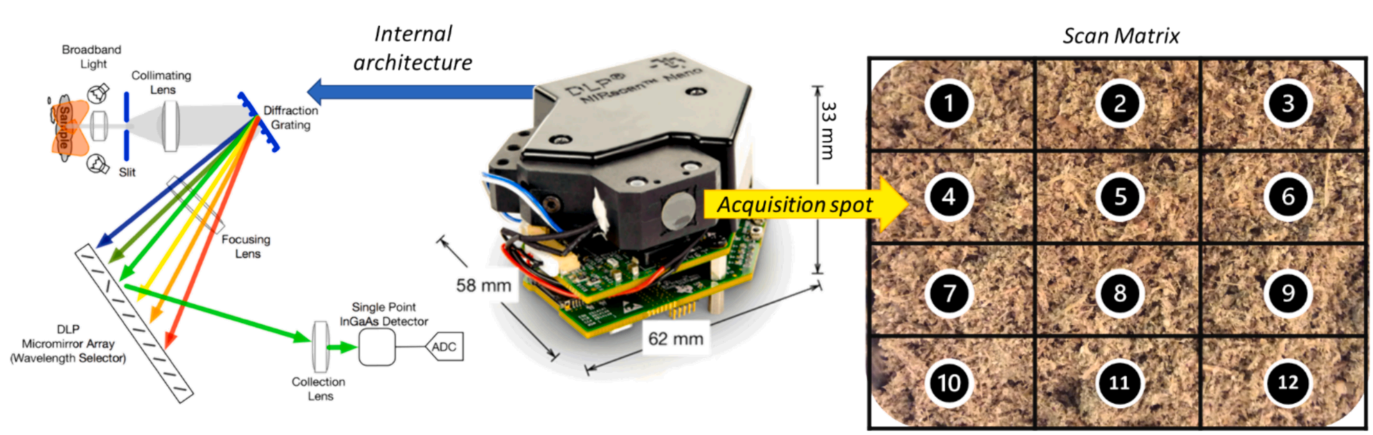

Fig. 4. Description of the NIR device internal architecture and data acquisition.

originating from undesired light scattering phenomena) (Oliveri et al., 2019). Finally, the bi-dimensional data matrix was mean-centred column-wise, to minimize local differences between wavelengths (Biancolillo & Marini, 2018).

At first, models were developed using latent variables (LVs) by means of the Partial Least Square Regression (PLS) method. Such machine learning technique works maximizing the covariance among the NIR optical data and the reference analysis of DM and BMP providing more robust linear models. Finally, the performance of the models was assessed using metrics such as the Root Mean Square Error (RMSE), bias, coefficient of determination (R^2), and Residual Prediction Deviation (RPD), which account for the ratio between the standard deviation of the reference measurements and RMSE. An RPD between 1.5 and 2 indicates the capability of the model to distinguish low from high values of the response variable. When the RPD falls within 2 and 2.5, the model is capable of providing approximate quantitative predictions. Instead, an RPD higher than 2.5 indicates a high level of accuracy in prediction (Oliveri et al., 2020; Nicolai et al., 2007; Tugnolo et al., 2021).

By the end, the model performance was evaluated in calibration and in cross-validation (leave-more-out cross-validation using the Venetian Blinds method with 10 data splits and one sample per blind) in order to define the most suitable number of LVs (typically as little as possible in order do not burden to the complexity of the model) for maximizing the model reliability balancing good predictions and overfitting.

3. Results and discussions

3.1. Wet-chemical data analysis

Fig. 5 summarizes the descriptive statistics related to the reference analyses (DM and BMP) performed using the UNI EN ISO standard methodology on the 29 corn silage samples obtained from the 29 dairy farms employed for this study. The mean, median, interquartile range, the data range were represented in the graph together with the specific values of the mean, standard deviation (std), number of samples and the minimum and maximum in the legend. The potential and extreme outliers (observations located beyond the length of the data range whiskers) were also statistically represented. By default, a potential outlier is defined as a value that exceeds 1.5 times the interquartile range from the lower or upper edge of the box. Overall, similar results were obtained comparing the two analyses in terms of sample distribution suggesting a foreseeable correlation between DM and BMP. For this reason, since the data cover a reasonable range (Triolo et al., 2014) which could be representative of the sample's real conditions, the extreme values were not considered as outliers and were used for the model building.

The plots matrix shows the Pearson correlation analysis performed on DM and BMP. Histogram distribution plots of the variables are presented along the matrix diagonal while the correlation scatter plots of variable pairs are shown in the off-diagonal. The Pearson correlation showed an highly significant (p-value < 0.001) correlation coefficients (r) equal to 0.98 (Fig. 6). Such highly correlation is also confirmed by Mayer (2015) who investigated the ability to predict the BMP of wet maize silages from the substrate biochemical composition (volatile solids, ash, cellulose, hemicellulose, acid detergent lignin, crude proteins, elemental carbon, fat, starch). Results from Mayer highlighted that Total Solids (TS) content of the substrate mainly influenced the BMP, not having the individual biochemical fractions a major impact on BMP (Mayer, 2015).

3.2. NIR spectra exploration and regression

Fig. 7a shows the raw spectra (902–1701 nm) obtained from the proposed device. At a glance, no differences were identified on the NIR spectra chemical assignments from the 29 sampling sites. The main band appears around 1450 nm, which is strictly related to the water absorption bands due to the stretching of the O–H first-overtone (Tugnolo et al., 2021). While, between 1100 and 1250 nm, the characteristic bands related to the second stretching overtone of C–H thanks to the presence of lipids and carbohydrates were noticeable (Beć et al., 2022).

PCA was performed on the pretreated (Der 1 transform) NIR spectral data to explore and evaluate the sources of variability retained into the dataset (348 spectra). Fig. 7b and 7c show the joint interpretation of PCA outcomes (Scores and Loadings) describing (in the Loading plot) the impact on the first 3 PCs (cumulative explained variance almost 93 %) of the original variables (wavelengths). As noticeable, especially from the right spectral tail, a high impact of the wavelengths around 1600 and 1700 nm affect the first 3 sources of variation. Such right tail impact shows a temporal clustering of the data mainly described by the major component (PC1). Since the data were collected in 4 sampling dates from 29 different dairy farms (within a radius of 50 km, Fig. 1), particular temporal effects were not expected as major sources of variation. Therefore, such part of the spectrum has been ascribed as a source of non-information linked to a drift of the instrument that had to be removed to make room for the chemical information linked to the parameters considered (DM and BMP). Therefore, spectra were cut from 1050 nm to 1570 nm to minimize any possible source of noise coming

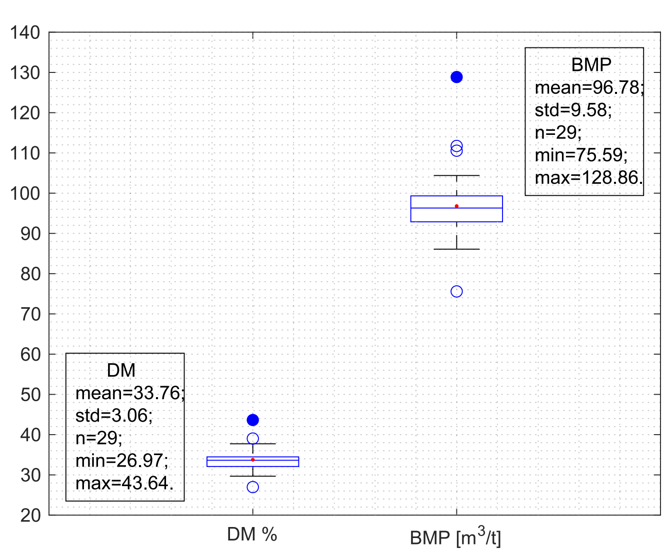

Fig. 5. Descriptive statistics of corn silage qualitative dry matter (DM) and biochemical methane potential (BMP).

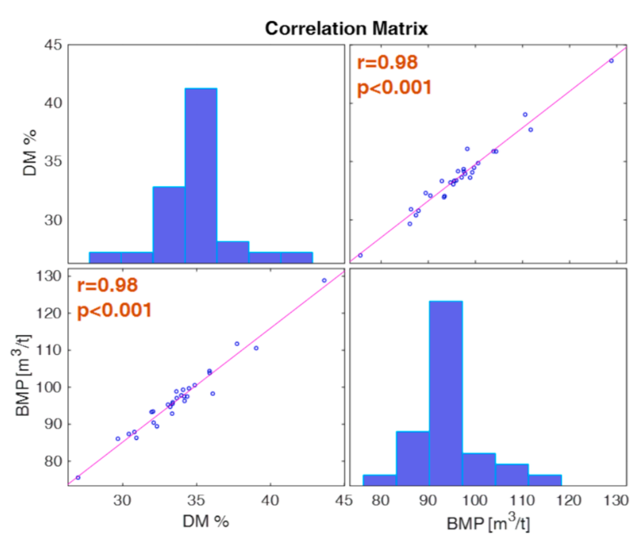

Fig. 6. Pearson correlation analysis and frequency (on diagonal) plot for DM and BMP.

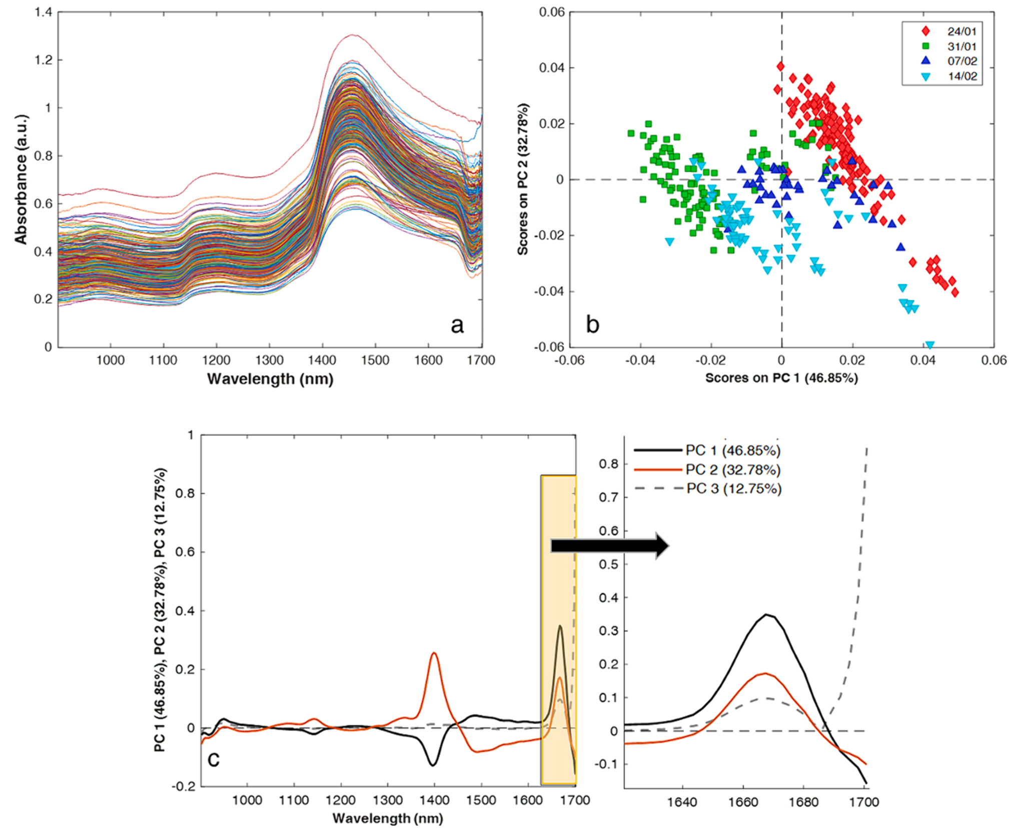

Fig. 7. Raw spectra (a) and PCA outcomes (score plot (b) and loadings plot (c)) of the 3 lower order components using Der1 transformed data.

from the tails.

Fig. 8 shows a second PCA performed on pretreated (Der1 transformed) spectral data from 1050 nm to 1570 nm. The first 2 components describe about 95 % of the total variability. To represent at best the data distribution, each reference value has been used as label for each spectrum. Therefore, for each sample, the value of DM and BMP were used 12 times in order to be associated with the 12 acquisitions performed on each sample. Consequently, PCA scores were labelled according to values of DM (Fig. 8a) and to BMP (Fig. 8b) showing an interesting color trend from negative to positive values of PC2. Such behavior demonstrated (in an unsupervised manner) the capability of the optical device to pick the optical variation related to the chemical information of the two reference parameters (DM and BMP). Fig. 8c shows the loading plot with the contribution of the original variables to describe the scores trend related to the variation of DM and BMP. No outliers were identified into the dataset. For this reason, considering the homogeneity distribution of the samples and the possible lack of outliers, each group of 12 spectral replicates were averaged (to reduce any possible experimental noise) to obtain 1 spectrum to be associated with the reference analysis (DM and BMP) during the modelling phase.

Fig. 9a and 9b show the averaged pretreated (Der 1 transformed) spectra labelled according to DM and to BMP. The effect of the first derivative visually enhances the color trend described by the colorbar (from dark blue to dark red). Indeed, such row-wise preprocessing was able to minimize the scattering effects related to the physical structure of the samples highlighting the optical ranges where a variation in the percentage of DM and BMP is evident (around 1300–1420 nm). Such visual interpretation of the averaged spectra confirms the results obtained from Fig. 8c where a large contribution of the original variables around 1300–1420 nm was recognized. This confirmed again the potential capability to build a regression model for the DM and BMP prediction thanks to the high correlation between DM and BMP and the good variation retained into the data collected in the 29 sampling sites.

Consequently, leveraging the promising outcomes obtained from this preliminary phase of data processing, regression models employing the PLS approach were established to predict DM and BMP using the NIR data. Fig. 10 (reference vs. predicted) shows the figures of merit utilized to assess the quality of the models for both DM and BMP (Fig. 10a and 10b, respectively). Concerning R^2 and RMSE, It is noteworthy to highlight the good proportion of variability in the dependent variable that is explained by the independent variables in the PLS models both in calibration and cross validation ($R^2_C = 0.92$ and $R^2_{CV} = 0.80$ for DM and $R^2_C = 0.90$ and $R^2_{CV} = 0.75$ for BMP), and the reasonable prediction error ($RMSE_{CV} = 1.38$ and 4.76 for DM and BMP, respectively).

Results for DM are comparable with other works. Liu and Han (2006) using a benchtop NIR system (Spectrum One NTS, PerkinElmer) over the 1000–2500 nm wavelength range reported R^2_C and $RMSE_C$ values for DM respectively of 0.96 and 12.90 with fresh samples of maize silage. Berzaghi et al. (2005), in a study aimed to evaluate the feasibility of using a portable spectrophotometer (Zeiss Corona 45, 960–1700 nm) to analyze corn silage without any prior sample preparation got a R^2 of 0.87 for DM. More recently, Feng et al. (2023) using handheld NIR spectrometers (Si-ware NeoSpectra scanner, 1350–2550 nm) on undried, unground corn silage samples obtained R^2_{CV} and $RMSE_{CV}$ values respectively ranging between 0.94–0.96 and 1.68–2.16 according to the scanning method applied. The worst results referred to a stationary scan foresing four 4 s measurements with the instrument in contact with the forage, while the best ones involved sliding the instrument across the sample during scanning.

Calibration statistics for BMP were better than those obtained by Mayer et al. (2015) in NIRS performed on wet maize silages using a benchtop spectrometer (Bruker MPA) operating in a wavelength range of 780–2586 nm (0.90 vs. 0.85 and 2.95 vs. 9.50 respectively for R^2_C and $RMSE_C$). In cross validation we obtained a slightly lower R^2 (0.75 vs. 0.83) compared to Mayer (2015), but a far lower RMSE (4.76 vs. 10.0) was computed giving the model a higher accuracy of prediction.

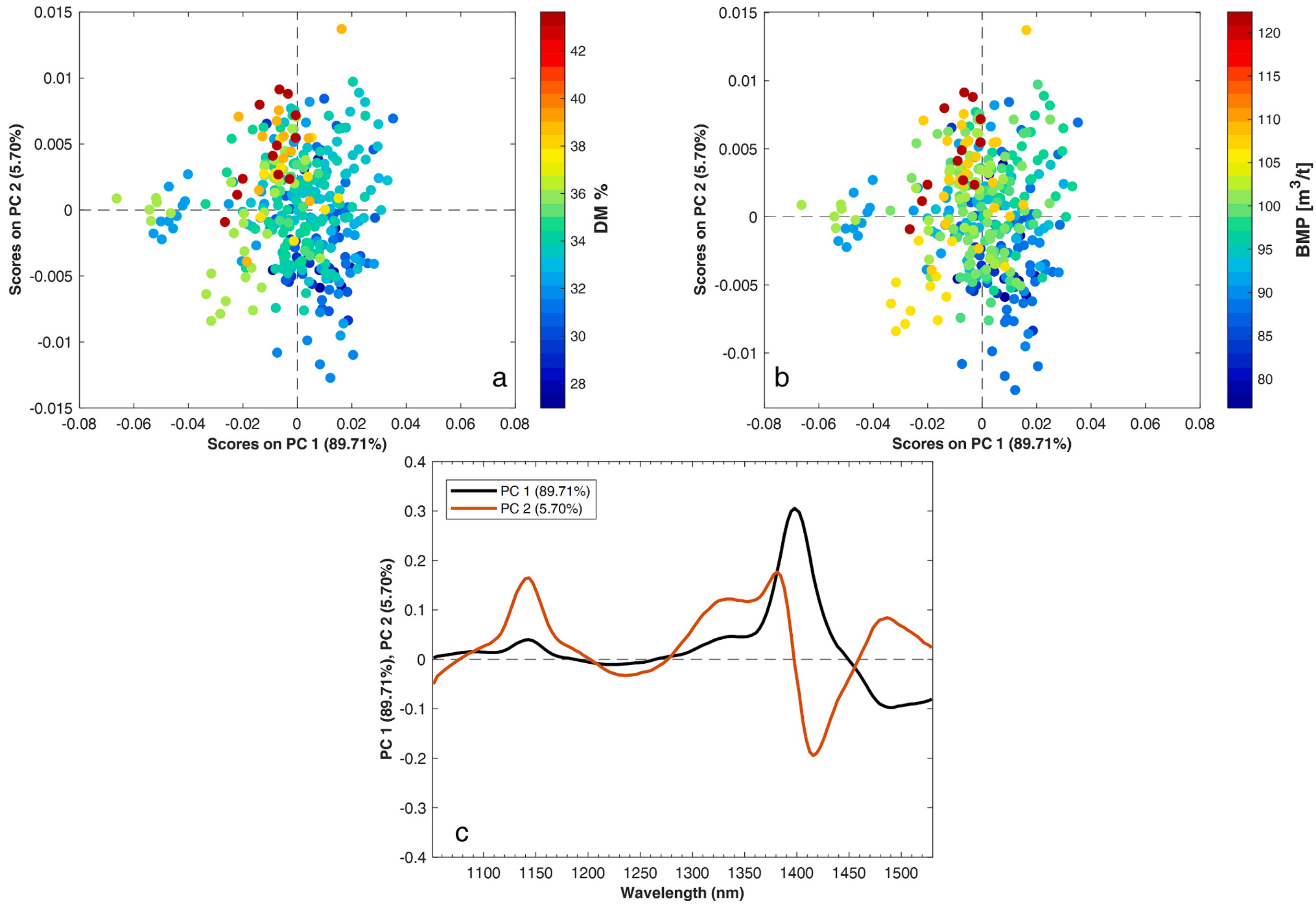

Fig. 8. PCA outcomes of the 2 lower order components using De1 transformed data. (a) scores labelled according to DM values, (b) scores labelled according to BMP values, (c) loadings.

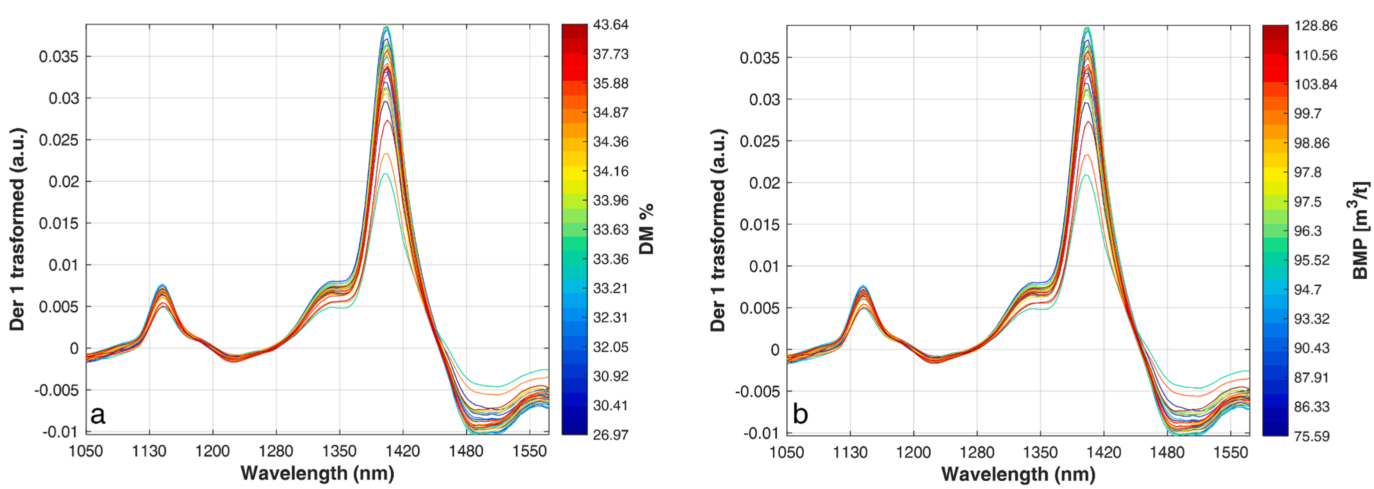

Fig. 9. Pretreated spectra (Der 1 transformation) of grinded corn silage labeled according to DM (a) and BMP (b) analysis.

Furthermore, RPD values surpassing the threshold of 2 serve as compelling evidence of the model's efficacy in rapidly assessing the qualitative attributes of corn silage. The salient findings, gleaned from a relatively modest sample size of 29 specimens procured from diverse sampling locations, validate the practical applicability of this methodology within operational contexts. Moreover, it underscores the potential for refining predictive models through the expansion of the calibration dataset. Despite the limited number of samples, the incorporation of heterogeneous farms spanning a broad geographical area (encompassing a 50 km radius) enriches the diversity of the collected samples, thus optimizing the information amenable to modeling concerning the parameters of interest.

An additional noteworthy aspect of the models' predictive prowess emanates from the homogenization process achieved during mixing and grinding, ensuring a representative depiction of the chemical-physical conditions inherent to each silo panel within every farm.

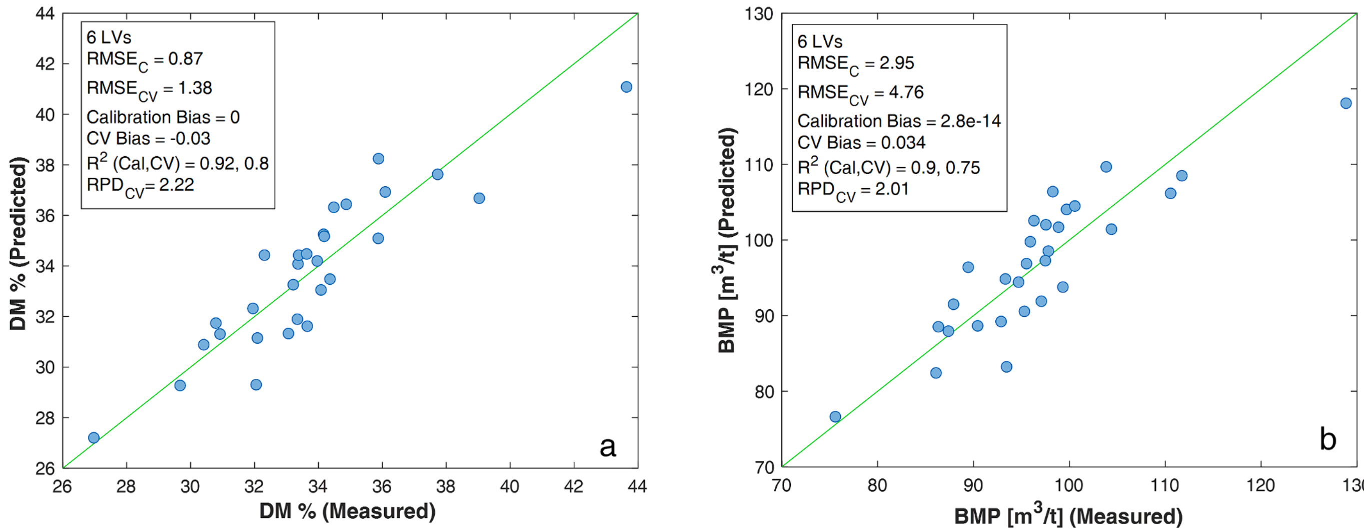

Fig. 10. Figure of merit of reference vs. predicted for DM (a) and BMP (b).

Consequently, these findings affirm the viability of leveraging a cost-effective NIR spectrometer for the direct estimation of DM and BMP on-farm, spreading the access to technology previously deemed economically prohibitive, impractical and time-consuming. This expedited alternative to conventional methodologies streamlines on-site measurements, mitigating the necessity for off-site laboratories and extensive sample preparation. Consequently, it fosters real-time feedback integration into farm advisory systems, thereby facilitating more informed and timely decision-making processes.

4. Conclusions

In this work, a cost-effective handheld NIR spectrometer for fast measurements DM and BMP at the farm level with minimal preparation of the samples has been proposed to optimize the management of biogas plants, enabling fast feedback in farm advisory systems. Leveraging advancements in technology, the study confirmed existing correlations between DM and BMP, as demonstrated by analyzing corn silage from 29 different farms scattered in northern Italy, obtaining a highly significant Pearson's correlation. The study showcased the feasibility of using NIR techniques to develop an on-site optical system capable of reducing the cost and time required for the analysis of DM and BMP (RMSECV = 1.38 and 4.76, respectively). By leveraging the entire NIR range, the proposed device exhibited extreme versatility, allowing for the prediction of various qualitative features on different biological matrices, such as corn silage. However, it is essential to acknowledge certain limitations. The relatively modest sample size of 29 specimens may limit the generalizability of the findings, suggesting the need for further validation with larger and more diverse datasets. Furthermore, variations in sample preparation methods across different farms could potentially impact the results, emphasizing the importance of standardization and consistency in sampling procedures. Nevertheless, the potential for scalability can be emphasized, indicating that the success of the NIR device in estimating DM and BMP for corn silage suggests its potential applicability to other agricultural feedstocks and biomasses. This scalability opens up opportunities for broader adoption across different farming and biogas production contexts. To further expand the implementation of the proposed NIR technology for detecting DM and BMP in corn silage samples, future works could integrate the device into a portable case along with a grinding machine to homogenize the samples and reduce scattering effects, enabling on-site measurements. Additionally, efforts should focus on improving the accuracy of the DM and BMP prediction models by increasing the number of samples within the calibration dataset.

Overall, this study highlights the potential of NIR spectroscopy as a valuable tool for optimizing biogas plant management and facilitating informed decision-making processes in agricultural biogas production. Further research and development in this area promise to enhance the practicality and reliability of on-farm NIR measurements, paving the way for more efficient and sustainable agricultural practices.

CRediT authorship contribution statement

Francesco Tangorra: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **Alessio Tugnolo:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ze'ev Schmilovitch:** Methodology, Supervision, Writing – original draft, Writing – review & editing, Formal analysis. **Aldo Calcante:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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