Effects of measurement technique and sample preparation on NIR spectroscopy analysis of livestock effluents and digestates

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Abstract

The purpose of this study was to evaluate different measurement technique as sample treatments, probes used and sample temperature during NIR scan, in order to obtain accurate and reproducible values of Total Solids (TS), Total Kjeldahl Nitrogen (TKN), Total Ammonia Nitrogen (TAN) and Volatile Fatty Acids (VFA), contained in dairy and swine slurry and digestate. In particular, three sample treatments were tested as filtration, homogenization and a raw control, two types of probes (petri dish and optical fiber) and three different sample temperature during NIR scan (10-25-35°C). We studied the possible significant influences on predictive capability of analyte contents and which experimental condition, allow to obtain the best results. The results show that spectral acquisition through petri dish was more accurate respect to optical fiber. The better sample treatment was represented by filtration. We did not observe any effect of the temperature in the reported range.

Introduction

NIR spectroscopy was applied successfully for the slurries or digestates analysis to predict the content of Total Solids (TS), Total Kjeldahl Nitrogen (TKN), Total Ammonia Nitrogen (TAN) [1] [2] [3] and Volatile Fatty Acids (VFA) [4] [5]. Currently, there is not a standard protocol for NIR analysis of livestock effluents and digestates.

In the case of TS, TKN and TAN, some authors used the raw samples of dairy or swine slurry, poured into a polyethylene bag and scanned in reflectance at a controlled temperature [1] [2] [3]. The samples were scanned between 23-27°C [2], or at room temperature [1], or at 4°C [3]. In these works were obtained models with r^2 between 0.79 and 0.92. The raw samples of swine slurry were analyzed also in transflectance with an optical fiber. The results obtained on TS and TKN were similar respect to those cited above, while for TAN was poor [6]. VFA content was usually measured for the digestates, but it could be interesting to measure this parameter in dairy and pig slurry as they are a commonly used for the feeding of biogas plants. Several studies showed a satisfactory application of the NIRS to predict the digestate VFA content in different experimental settings. Significant PLS-prediction models for total VFA, acetic and propionic acid were reported with TENIRS System on homogenized samples scanned in transflectance [5]. On the contrary using a raw digestate samples poured into a cuvette and scanned in reflection at 35-40°C, NIR could predict the content of total VFA (r^2 0.92-0.94), not the acetic and propionic acids [4].

The cited works had different approaches to define experimental protocol, therefore the aim of this preliminary study was to evaluate the influence of sample treatment, probe used and sample temperature during NIR scan in order to obtain accurate and reproducible values of TS, TKN, TAN and VFA contained in dairy and swine slurry and digestate. We studied the possible significant influences of the different experimental conditions tested, on predictive capability of analyte contents.

Material and Methods

Field experiment

A total of 36 samples were collected (12 each for dairy slurry, swine slurry and digestate), considering the number of samples adequate for a preliminary assessment [8]. Each sample was divided into three subsamples: not treated (raw slurry), homogenized (50-100μm) with a homogenizer (Ultra Turrax IKA ® T18 ™) and filtered, with 1 mm mesh filter. The reference chemical analysis, were performed on all fresh raw and filtered samples. Not analysis were carried out on homogenized samples because we assumed that they had the same composition of the corresponding raw sample. Total solids (TS)

content of samples were determined according to standard procedures [8]. TKN and TAN were determined using the analytical method for wastewater sludge on fresh samples [9]. Extraction and quantification of total VFA was made by titration [10]. The Tables 1 and 2 show the samples mean, standard deviation (SD) and the measuring range.
The spectra were acquired using an FT-NIR Buchi Flex-N-500 spectroscophotometer, working in a spectral range between 1000-2500nm, with a resolution of 2 nm and 32 scans per spectrum. Each subsample (raw, homogenized and filtered) was analyzed in transflectance (optical path of 0.3mm) with two different reading-setup of NIR spectrophotometer, petri dish and the optical fiber probe, at 3 temperatures (10-25-35°C). The temperature is important because can affect the spectral shape, shifting the peaks [11].

Table 1. Composition of raw samples

RAW	Digestate		Dairy Slurry		Swine Slurry	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
TS (%)	5.79 ± 1.70	3.43 - 8.62	9.01 ± 1.67	6.34 - 11.24	4.90 ± 4.87	1.41 - 19.09
TKN (g l-1)	3.93 ± 0.96	2.53 - 5.54	3.31 ± 0.54	2.52 - 4.13	3.82 ± 2.11	1.28 - 9.27
TAN (g l-1)	2.22 ± 0.88	0.72 - 4.41	1.53 ± 0.30	1.15 - 2.13	2.24 ± 0.96	0.39 - 3.59
VFA (mg l-1)	1186 ± 1295	213 - 4692	7762 ± 2476	2977 - 12466	4451 ± 4406	469 - 15234

Table 2. Composition of filtered samples

FILTERED	Digestate		Dairy Slurry		Swine Slurry	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
TS (%)	3.61 ± 0.80	2.49 - 4.68	5.24 ± 0.94	3.69 - 6.45	2.94 ± 2.71	0.53 - 10.35
TKN (g l-1)	3.74 ± 0.94	2.18 - 5.33	3.16 ± 0.65	2.17 - 4.12	3.64 ± 2.16	1.22 - 9.14
TAN (g l-1)	2.21 ± 0.84	0.84 - 4.20	1.56 ± 0.28	1.26 - 2.19	2.23 ± 1.05	0.36 - 4.10
VFA (mg l-1)	1399 ± 1495	157- 5155	7887 ± 1890	3727 - 10416	4482 ± 4622	155 - 15386

Modelling
The spectra obtained were processed with CAMO Unscrambler 9.7. A Partial Least Squares Regression (PLS) was performed by setting categorical variables (slurry, probe, treatment, temperature levels) to study the possible correlation of the spectra with the analytes content of the manures, that are TS, TKN, TAN and VFA.
The PLS was assessed both in calibration and cross-validation (leave one out). A separate PLS regression was developed from the NIR scan data to predict each nutrient in each type of manure [1] [2].The PLS was performed by dividing the spectra into subgroups according to different treatments (raw, homogenized, filtered) and to the measurement techniques probes (optical fiber and petri dish).
Within the experimental plan, the best combination of treatment and measurement condition allowing to better predict the analyte content of TS, TKN, TAN and VFA were identified looking for the highest r^2 (coefficient of determination), the lowest RMSECV (Root Mean Square Error in Cross validation), and the highest RPD, which is the ratio of standard deviation (SD) to the RMSECV. RPD it is a meaningful measure of NIRS prediction [1].
No spectral pre-processing technique was applied to raw data, to allow the direct comparison of the different experimental conditions considered. Indeed, a huge variety of mathematical treatments used in specific methods, can be applied in NIR spectral analysis of slurries [1] [6] depending on goals, instruments or experimental conditions, in seek of the best prediction capability of developed models.

Results
The main results of the PLS models are reported in Table 3. On the whole, the models relating to swine slurry showed a greater accuracy, as in [1], respect to those concerning dairy slurry, providing reliable models (typically with $r^2>0.9$ and RPD>3) for all the analytes and conditions considered. For the digestate and dairy slurry it was even not possible to define any correlations between the NIR

spectra and the level of the parameters of interest. In general, RPD and r^2 values belonging to dairy slurry were lower relating to swine slurry and digestate. We can assume that the analysis conducted on dairy slurry referred on a more complex substrate rich in vegetable fiber and coarse material that hinders the optical path.

Table 3. Results of PLS models

	TKN			TAN			VFA			TS		
Dairy slurry	r^2	Rmsecv (g/l)	RPD	r^2	Rmsecv (g/l)	RPD	r^2	Rmsecv (mg/l)	RPD	r^2	Rmsecv (%)	RPD
Filtered OF	0.79	0.29	2.26	0.91	0.08	3.55	0.91	553	3.41	0.87	0.33	2.85
Filtered P	0.81	0.27	2.42	0.91	0.08	3.55	0.91	552	3.42	0.92	0.25	3.76
Raw OF	0.54	0.35	1.54	0.49	0.21	1.43	0.17	2263	1.09	0.11	1.52	1.10
Raw P	0.89	0.17	3.18	0.91	0.09	3.33	0.8	1674	1.48	0.75	0.8	2.09
Homog. OF	0.8	0.22	2.46	0.75	0.14	2.14	0.55	1674	1.48	0.32	1.19	1.40
Homog. P	0.91	0.15	3.60	0.87	0.11	2.73	0.9	807	3.06	0.6	0.92	1.81

	TKN			TAN			VFA			TS		
Swine slurry	r^2	Rmsecv (g/l)	RPD	r^2	Rmsecv (g/l)	RPD	r^2	Rmsecv (mg/l)	RPD	r^2	Rmsecv (%)	RPD
Filtered OF	0.94	0.50	4.31	0.95	0.22	4.78	0.95	1025	4.51	0.88	0.89	3.05
Filtered P	0.94	0.51	4.27	0.95	0.23	4.57	0.98	693	6.67	0.88	0.89	3.05
Raw OF	0.89	0.69	3.06	0.9	0.28	3.41	0.77	2140	2.06	0.87	1.76	2.77
Raw P	0.92	0.59	3.58	0.83	0.36	2.66	0.96	912	4.83	0.95	1.03	4.73
Homog. OF	0.92	0.63	3.35	0.92	0.26	3.68	0.94	1107	3.98	0.9	1.58	3.08
Homog. P	0.92	0.60	3.52	0.95	0.21	4.55	0.97	1023	4.31	0.96	0.87	5.60

	TKN			TAN			VFA			TS		
Digestate	r^2	Rmsecv (g/l)	RPD	r^2	Rmsecv (g/l)	RPD	r^2	Rmsecv (mg/l)	RPD	r^2	Rmsecv (%)	RPD
Filtered OF	0.92	0.26	3.60	0.96	0.17	4.93	0.8	649	2.30	0.86	0.29	2.74
Filtered P	0.97	0.16	5.85	0.98	0.12	6.99	0.86	535	2.79	0.92	0.22	3.61
Raw OF	0.42	0.71	1.36	0.84	0.34	2.60	0.03	1231	1.05	0.54	1.12	1.52
Raw P	0.78	0.43	2.24	0.89	0.28	3.16	0.66	726	1.78	0.67	0.94	1.81
Homog. OF	0.89	0.32	3.01	0.92	0.24	3.68	0.3	1046	1.24	0.88	0.58	2.93
Homog. P	0.93	0.25	3.85	0.97	0.14	6.31	0.78	592	2.18	0.93	0.47	3.62

The type of employed sample treatment influenced the robustness of the PLS models in dairy slurry and digestate, while had not a great effect on swine. Probably because this slurry was characterized by particles of smaller size, that do not hinder the optical path.

In filtrated samples of dairy slurry, robust models were obtained for prediction of TS, TAN and VFA (r^2>0.91 and RPD>3.41), while the regressions for TKN were less trustworthy (RPD<2.42). Although the levels of TKN were similar in all manures, as reported in Tables 1 and 2, the accuracy of the PLS models differed as described among different type of manures. This could probably reflect the degree of variability of the three datasets: swine slurry showed largest values of SD, while dairy slurry was characterized by a very low SD. Furthermore, the dairy slurry with respect to the swine slurry and the digestate was characterized by a lower homogeneity of the sample.

A high correlation between spectra and the measured parameters was found for filtration in swine slurry, even with r^2=0.98 and RPD=6.67 for VFA prediction. Similar results were obtained for digestate samples with the exception of VFA, which were present in small amounts in these samples as reported in Table 2. In this study, the models on the digestate did not achieve the accurate results obtained by other authors [4] [5] probably because there was less variability in the samples.

Measurements of TS, TKN, TAN and VFA conducted on homogenized samples were slightly less effective of the filtrated ones according to the built models. In some cases the predictive capability of the homogenized samples is better than other treatments, as the TKN for the dairy slurry, in accordance with [3] and the TS for swine slurry in line with [1]. The obtained outcomes witnessed that raw samples showed the worst performances in measuring analyte when compared to the other treatments. Even if in dairy slurry TKN and TAN prediction was reliable for the raw samples.

Probes. The optical fiber rather than petri dish also greatly influenced the reliability in measuring analyte levels in the samples. In general, for all the slurries and for every sample treatment, spectral acquisition through petri dish was more accurate respect to optical fiber. However the results obtained for TKN and TAN on swine slurry were robust for the two probes and in accordance with [6]. Temperature. In the work we acquired the spectra at three different temperatures. We did not observe any altering effect of the temperature in the reported range. Models built at 10-25-35°C respectively, provided really similar results in terms of r^2, RMSECV, RPD (data not shown). Of course, the temperature maintains a relevant effect on the model parameters and should be carefully considered in practical application.

Conclusion and perspectives

The results confirm the reliability of NIR spectroscopy for the analysis of complex substrates such as livestock slurries and digestates, although the assessment of analytes content, may benefit of a sample treatment. Spectral acquisition through petri dish was more accurate than optical fibre. The better sample treatment was clearly represented by filtration. With this treatment there were not big differences between the optical fiber and petri dish. Based on the chemical analysis, TAN and VFA content is very similar between filtered sample with the corresponding raw. This could make it possible the application of this treatment in order to obtain better models, referring to the raw sample. The TKN content of the filtered sample is lower than the raw one, but the models improvement might compensate the loss of information introduced with filtration. While, for the TS content, the difference between filtered and raw is greater than the other analytes. We did not observe any altering effects of the temperature in the reported range.

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