RAPID ANALYSIS OF HOG MANURE USING VISUAL AND NEAR-INFRARED REFLECTANCE SPECTROSCOPY

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INTRODUCTION

Problem

The intensification of the animal production since 1950, resulted in an increased production of animal wastes. At the same time the intensification of the crop production was obtained by the application of tons of artificial fertilizer. The combination of both has led to the over-fertilization of the arable land with leaching of nutrients to and pollution of the surface and groundwater as a consequence. To tackle this problem the European council stated in 1991 its 'Directive 91/676/EEC concerning the protection of waters against the pollution caused by nitrates from agricultural sources', limiting the amounts of manure to be produced and of fertilizer to be applied.

Although most farmers know that manure has a fertilizer value, they are not eager to radically decrease their artificial fertilizer use, when applying manure. The main reason for this behaviour is their fear for productivity losses resulting from lack of a certain nutrient due to the unknown and variable composition of the manure. Moreover, matching fertilizer application to the soil reserve, measured by soil sampling, and the crop needs, found in literature, can be easily done with artificial fertilizer since the composition is known and guaranteed. Therefore, manure can only become a viable alternative for artificial fertilizer if the composition is known. Traditionally, this composition determination is done by wet chemical analysis, which takes several days and is quite expensive. Moreover, only the mean composition, by mixing several sub-samples, is determined for a whole storage facility. Unfortunately, manure consists of solid particles, containing most of the organic matter, phosphorus, calcium and magnesium, in a liquid matrix, containing most of the nitrogen and potassium, which tend to separate during storage due to their different densities (Bries *et al*., 1997). Since the fluid is pumped up more easily than the solids, the first tanks hauled out of a storage facility will be more diluted and have a lower phosphorus to nitrogen ratio than the last tanks. Use of the mean composition for a complete storage facility for each of these tanks can therefore result in a very poor nutrient management.

Sampling at haul-out time would give a more reliable composition for each tank resulting in a better management. However, the time delay induced by the wet chemical analysis makes good manure nutrient management very difficult since the land application may be completed before the sample results are known (Milmier *et al*., 2000). A rapid and accurate on-farm analysis technique could tackle this problem, converting manure into a viable alternative for the larger part of the artificial fertilizer use. This would promote a fertilizer management that is economically and ecologically sound.

Literature review

During the last decades near infrared (NIR) reflectance spectroscopy has become a rapid and valuable alternative for compositional analysis of agricultural products (Shenk *et al*., 1992). Milmier *et al.* (2000) calibrated a scanning monochromator ranging from 400 to 2498 nm for compositional analysis of swine lagoon effluent, liquid swine pit manure and solid beef feedlot. They obtained good predictions for total solids, total Kjeldahl N, ammonium-N and K, while the prediction of P needed further research. To obtain these promising results they unfortunately had to remove between 2 and 24% of the samples as outliers. Reeves and Van Kessel (2000) tested two fibre-optic based scanning monochromators to determine ammonium-N, moisture, total C and total N in dairy manures. They reported that a scanning monochromator ranging from 400 to 2300 nm can be a viable alternative to other Quick Tests on the market, whereas one using only the 400 to 1098 nm region is not. Malley *et al.* (2002) calibrated a monochromator ranging from 400 to 2500 nm with a sample cell in transflectance mode for the analysis of hog manure and manure-amended soils. They obtained very good results for a set of 64 manure samples from 7 storage facilities, but were less successful for a set of 75 manure samples from 25 facilities.

Objective

While the previous work, described in literature, showed the value of NIRS for the analysis of manure using a lab instrument, the purpose of this research was to determine the feasibility of a mobile and - if possible - cheap, diode array instrument for the rapid and accurate on-site (or even on-line) analysis of hog manure.

METHODOLOGY

Manure samples

A set of 169 chemically analyzed hog manure samples was selected to have an as uniform distribution of constituent concentrations as possible over a wide range (Table 1). All samples were collected in the spring of 2003 at different farms in Flanders, Belgium. Approximately 1 L of each sample was collected and mixed. All samples were stored in a closed container at 4℃ from the time of sampling until the time of analysis to minimize the change of the components of interest by microbial and chemical reactions (Reeves & Van Kessel, 2000).

Chemical analysis

Sampling and analysis for pH, dry matter, organic matter, total Kjeldahl nitrogen, ammonium nitrogen, phosphorus, potassium, sodium, calcium and magnesium were performed by the Soil Service of Belgium (Heverlee, Belgium) using the official analysis methods described by the Flemish institute for technological research (Anonymous, 2002). The ammonium nitrogen content was spectroscopically determined after attenuation, while the total nitrogen content was determined by the Kjeldahl method. The phosphorus content was determined by incineration of the sample at 550 ℃, solution of the ashes in HNO_3 and spectrofotometrical determination of the concentration.

Spectroscopic analysis

The samples were scanned using a Zeiss Corona VISNIR 1.7 diode array instrument equipped with an OMK measuring head. This measurement head with its 45° position of the detectors has an effective measuring area of 20 mm, which is very interesting for a heterogeneous material such as manure. This instrument has no moving parts and can therefore be used on mobile machinery such as a combine (Reyns *et al.*, 2001). The samples were scanned from the bottom in reflectance mode from 400 to 1710 nm. Before filling the cup, the manure was stirred with a magnetic stirrer and when necessary also with the sampling pipette.

Statistical analysis

The coefficients of correlation (r) between the different constituents (Table 2) and between the constituents and wavelengths were calculated and plotted to investigate whether there is a (common) spectroscopic basis for the prediction of the different variables. Based on this analysis the constituent variables were divided into two groups of variables having the same shape of the spectra-constituent correlation plot and one independent constituent variable, namely pH.

The development of the NIR calibrations was performed using Partial Least Squares Regression (PLS) in The Unscrambler 7.8 (Camo Inc.) based on a cross-validation with nine segments, corresponding to the nine measurement days. This approach corrects for the bias caused by the day-specific measurement conditions, because the resulting calibration is the mean of nine different calibrations where all samples measured on a certain day are either in the calibration set or in the test set. The number of latent variables was chosen based on the F test statistic.

To investigate the feasibility of a cheap sensor measuring only a few wavelengths, a multiple linear regression was performed between a few selected wavelengths and the constituent data. Although a lot of powerful, but often complex, variable selection methods are proposed in literature, a very simple approach was applied here. For each constituent the wavelengths for the MLR were selected as those corresponding to the tops of the peaks on the X regression coefficients plot for the PLS regressions. This approach was only possible because the regression coefficient plots were rather smooth. It was also investigated whether one or more of these variables could be removed without a significant loss of prediction performance. The 'best' model was selected based on the r and RER results for full cross validation and compared to the PLS results. After variable selection and calibration the useful accuracy of this rapid on-site manure composition sensor was investigated.

For more details the reader is referred to Saeys *et al.* (2004).

RESULTS AND DISCUSSION

Range of the calibration data set

The reliability of a NIR spectroscopic calibration will be restricted to the range of constituent values and the variation in measurement conditions taken into account during calibration (Williams, 2003). The basic statistics for the different constituents of the 169 collected manure samples used in this study are listed in Table 1. The sample consistency varied from that of water with few suspended solids (8.58 g L^{-1} DM) to that of highly viscous slurry (186.20 g L^{-1} DM). The constituent concentrations ranged from 10 fold for K to 77 fold for P. For pH there was only a range of 2.6 units, but this corresponds to a proton concentration range of 398 fold, because of the logarithmic scale of pH. Thus, the dataset included a large amount of compositional and physical variation, justifying this feasibility study for rapid on-site analysis of hog manure based on this set.

Table 1

Basic statistics for the sample set as determined by wet chemical analysis

Constituent	Min.	Max.	Mean	SD
pH	6.100	8.700	7.940	0.250
Dry matter (DM)	8.580	186.200	64.482	38.878
Organic matter (OM)	2.890	139.330	42.283	27.894
Total N	0.767	18.515	7.594	3.197
NH4-N	0.149	11.428	5.025	2.230
P	0.088	4.098	1.274	0.866
K	0.857	8.540	4.136	1.588
Na	0.096	2.055	0.903	0.371
Ca	0.159	8.173	1.895	0.394
Mg	0.030	2.060	0.800	0.558

Compositional analysis was performed at the Soil Service of Belgium, Heverlee, Belgium.

Sample spectra

The effect of the variation in dry matter content is illustrated in Figure 1. A decrease in the dry matter content, corresponding to an increase in the moisture content, resulted in an overall increase in absorbance across the spectrum. In the NIR region (700-1700 nm), there is a broad peak around 1450 nm, resulting from the absorption by the O-H bonds of water. Strange enough, the relative height of this peak, compared to the overall absorbance across the spectrum, decreases when the dry matter content decreases. The higher overall absorptions can be explained by the reduction of the number of scattering particles. When there are less scattering particles present (lower dry matter content) the chance that the light is reflected to the detector decreases, resulting in an overall increase of the absorption. This is not a true absorption by molecular bonds, but it is just an increased loss of the light. On the other hand, samples with lower dry matter content typically have smaller particles (especially when they are the result of a mechanical separation process such as sedimentation), which can reduce the penetration depth and thus the path length of the light that is reflected to the detector. Therefore, the true absorption by the O-H bonds will be less pronounced, resulting in a relatively smaller absorption peak around 1450 nm. In the visual region (400 – 700 nm), there is a high overall absorption which is higher in the blue region than in the red region. This results in the dark brown to black color of the manure. The sample with a dry matter content of 28.55 g L^{-1} has an absorbance peak around 675 nm, which probably corresponds to chlorophyll. This is rather exceptional for hog manure, but very common for dairy manure (Reeves & Van Kessel, 2000), because green forages are rarely used in pig nutrition. However, the use of CCM (Corn Cob Mix) for sow feeding by some Flemish farmers probably explains the appearance of this chlorophyll peak in some samples.

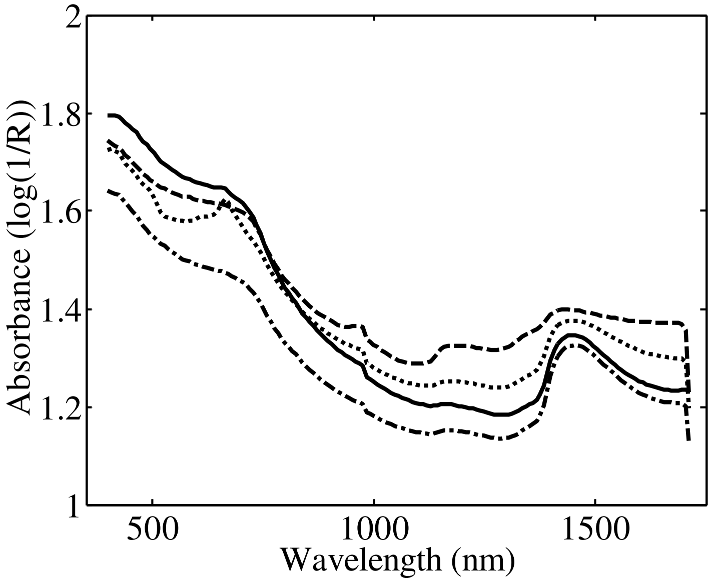

Fig. 1. Absorbance spectra (log(1/R)) of hog manure for different dry matter contents (g L^{-1}): 12.7 g L^{-1} (-----), 28.55 g L^{-1} (.....), 46.29 g L^{-1} (___) and 186.5 g L^{-1} (-.-.-.)

Spectral and constituent correlations

The correlation coefficients (r) between the constituent concentration and the absorbance at a certain wavelength were investigated for the different constituents and wavelengths. The correlation plots for DM, OM, P, Ca and Mg had a similar shape which differed from the similar shape for total Kjeldahl N, the ammonium N, K and Na. This could be expected since almost all the phosphate, Ca and Mg of the food not retained by the animal as live weight are voided in the dung, while the larger part of the unused nitrogen and almost all the potassium in an animal's food are excreted into the urine (Garner, 1957). The pH had a small positive correlation over the full spectrum, while the other constituents had negative correlations. Since only O-H, N-H and C-H bonds are absorbing in the NIR region, there is no spectral basis for the detection of cations, such as Na, K, Ca and Mg. Although cations can cause band shifts by influencing the hydrogen bonding (Shenk *et al.*, 1992), their high correlation with the spectra was probably caused by a correlation (e.g. due to chemical binding) with molecules that are spectrally active.

The correlations between the different nutrient concentrations measured by wet chemical analysis are listed in Table 2. The concentrations of OM, P, Ca and Mg were highly correlated to that of DM with r-values of 0.99, 0.88, 0.82 and 0.90 respectively, while the concentrations of NH4-N and K were highly correlated to the total N content with r-values of 0.94 and 0.87, respectively. Na has its highest correlation of 0.65 with K. These correlations explain the high spectral correlations for constituents like K and Mg that are known not to be spectrally active. The pH had no significant correlation with any of the other constituents and was therefore treated separately.

Table 2
Correlation coefficients (r) between nutrient concentrations

	DM	OM	P	Ca	Mg	total N	NH4-N	K	Na	pH
DM	1.00									
OM	0.99	1.00								
P	0.88	0.87	1.00							
Ca	0.82	0.78	0.93	1.00						
Mg	0.90	0.90	0.94	0.87	1.00					
total N	0.88	0.87	0.71	0.62	0.74	1.00				
NH4-N	0.80	0.79	0.64	0.56	0.68	0.94	1.00			
K	0.72	0.69	0.49	0.42	0.50	0.87	0.86	1.00		
Na	0.37	0.33	0.19	0.15	0.21	0.49	0.49	0.65	1.00	
pH	-0.16	-0.19	-0.21	-0.16	-0.23	-0.05	-0.01	0.16	0.21	1.00

Based on this analysis of the spectra-constituent and constituent-constituent correlations the constituents were divided into two groups, a DM-group of constituents that are highly correlated with the dry matter content (DM, OM, P, Ca and Mg) and an N-group with constituents that are correlated with the total nitrogen content (total N, NH_4-N, K and Na). The spectroscopic basis of the first group was expected to rely mostly on the O-H and C-H bonds, whereas the second group would rely more on the presence of N-H bonds.

It should be noted that rather high correlations and similar shapes were also found between members of the N-group and these of the DM-group. The correlation between Mg, P and NH4-N can probably be explained by the formation of struvite ($NH_4MgPO_4.6H_2O$) which has been found to cause settling of the mineral P and Mg in manure (Web & Ho, 1992), while the formation of apatite ($Ca_5(PO_4)_3OH$) could explain the high correlation between P and Ca. Since there is far more NH_4-N than Ca, Mg and P present in manure, almost all Ca, Mg and mineral P precipitate while a large part of the NH_4-N stays in solution.

Groupwise PLS calibrations

The above mentioned strong correlations between the constituent variables, suggesting a common spectroscopic basis, made it interesting to use PLS2 to model all the information of one group at the same time, reducing the noise sensitivity of the individual component calibrations (Bjørsvik & Martens, 1992). The results for these calibrations are presented in Table 3. The accuracy of the calibrations can be evaluated based on the RERcv (ratio of the data range to the standard error of prediction (SEP) for the cross validation). For total N, NH_4-N, K and OM this value lies between 8 and 12, showing a possibility of predicting quantitative data, while the values between 4 and 8 for DM, Na, P, Ca and Mg indicate a possibility to distinguish between high and low values (Milmier et al., 2000).

Table 3

a) DM-group PLS2 regression

		Calibration		Cross-validation	
Constituent	# LV's	r	RERc	r	RERcv
DM	5	0.815	8.41	0.780	7.77
OM	5	0.811	9.15	0.776	8.50
P	5	0.817	7.59	0.774	6.90
Ca	5	0.805	8.15	0.762	7.44
Mg	5	0.816	6.63	0.758	5.87

b) N-group PLS2 regression

		Calibration		Cross-validation	
Constituent	# LV's	r	RERc	r	RERcv
Total N	11	0.887	12.06	0.844	10.31
NH_4-N	11	0.867	10.19	0.819	8.79
K	11	0.886	10.45	0.836	8.79
Na	11	0.653	7.00	0.497	5.97

LV: number of latent variables (linear combinations of X variables)
r: correlation coefficient
RER: ratio of the concentration range over the standard error of cross validation (RERcv) and calibration (RERc)

MLR calibrations

The results of the Multiple Linear Regressions with the selected variables based on the X regression coefficients plots of the Partial Least Squares Regressions are presented in Table 4. Comparison of Tables 3 and 4 shows that these calibrations based on only 5 to 12 wavelengths are approximately as useful as those based on the whole spectrum using 4 to 11 latent variables. Combining the correlation between measured and predicted values (r) and the ratio of the data range to the standard error of prediction (RER) we can conclude that valuable quantitative predictions can be made for total N, NH_4-N, K and OM based on respectively 9, 12, 12 and 7 measured wavelengths in the spectral range of 400 to 1700 nm. For DM, Ca, P and Mg approximate predictions can be made using respectively 7, 5, 5 and 5 wavelengths in this range. The combination of a reasonably high RER with a low r for Na and pH indicate that further research is needed to obtain reliable calibrations for these constituents.

It should be noted that the calibrations for the members of the N-group use more variables (wavelengths for MLR, factors for PLS) than those for pH and the DM-group. This is probably because N-group calibrations use the absorbance peaks for the N-H bonds while the other factors rely more on shape effects originating from the combination of the particle size and loss of light effects as indirect indicators and the absorption by the C-H and O-H bonds as the direct indicators for the dry matter content.

Table 4
MLR results

MLR *Constituent*	*# var*	*Calibration* *r*	 *RERc*	*One-out cross-validation* *r*	 *RERcv*
DM	7	0.808	8.25	0.788	7.91
OM	7	0.806	9.10	0.787	8.72
P	5	0.819	7.62	0.806	7.38
Ca	5	0.799	8.03	0.782	7.74
Mg	5	0.827	6.83	0.814	6.61
Total N	9	0.822	9.75	0.798	9.24
NH4-N	12	0.854	9.74	0.821	8.86
K	12	0.863	9.60	0.834	8.77
Na	10	0.676	7.20	0.601	6.60
pH	6	0.463	7.56	0.367	7.18

Future work

There lies valuable information in the NIR region beyond 1800 nm, especially for determining NH_4-N (Reeves & Van Kessel, 2000). Since the Zeiss Corona VISNIR 1.7 only measures in the spectral region of 400 to 1700 nm, an instrument measuring up to 2500 nm might be able to predict the constituent concentrations more accurately.

During scanning the samples were not stirred, resulting in some sedimentation of the solids on the measurement glass. This sedimentation may have been an extra source of variation. Therefore, it should be investigated whether the mean of several scans on stirred or streaming manure results in a more homogeneous sampling and better calibrations.

The prediction of constituent concentrations highly depends on the correlation between spectrally active, directly measurable features and the constituents of interest. Manure is the result of food processed by an animal. Therefore, the constituent concentrations and their correlations for a manure sample will be the result of the correlations in the animal's nutrition changed by the way in which the animal processes it, and the sedimentation, digestion and hydrolysis of the solids during storage. These correlations will vary in the pit of one farm as well as between different farms. Therefore, a calibration based on samples from one or a few farms might be able to give an accurate prediction of the composition of an unknown sample of the same farm(s) (Malley *et al.*, 2002), but will probably do a poor job for samples coming from farms that were not represented in the calibration. Therefore, one should always be aware that a calibration will only be able to cope with the variation that is implicitly or explicitly taken into account during calibration.

The common way to determine the composition of manure, by taking several subsamples, mixing them and performing wet chemical analysis on this mean sample, only gives a mean composition for several tons of manure. Considering the highly heterogeneous character of manure it would be better to take more subsamples and analyze them separately. This may become possible by visual and near-infrared reflectance spectroscopy using a diode array instrument, mounted on a slurry tanker.

CONCLUSIONS

This study showed that valuable quantitative predictions can be made for total N, NH_4-N, K and OM, while DM, Ca, P and Mg can only be approximately predicted. For Na and pH the absolute values can be predicted roughly, but there is still a lot of variation in the constituent data which requires further research. One measurement, consisting of several scans, was enough to obtain a reliable spectrum. A cheap sensor measuring only a few appropriately selected wavelengths might be able to give comparable results.

Although further work will be necessary to optimize sample presentation and to refine procedures, mounting a diode array instrument on a slurry tanker can make environmentally and economically sound nutrient management possible.

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REFERENCES

[1] Anonymous (2002). *Bemonsterings- en analyseprocedures voor mest, bodem en veevoeder in het kader van het mestdecreet (BAM)*. www.vito.be.
[2] Bjørsvik H.-R. and Martens H. (1992). Data Analysis: Calibration of NIR instruments by PLS Regression. In: *Handbook of Near-Infrared Analysis* (Ed.) Burns D.A. and Ciurczak E.W. Marcel Dekker Inc., New York, pp. 159-180.
[3] Bries J., Vanongeval L. and Goppens G. (1997). Variaties in samenstelling van dierlijke mest: beïnvloedende factoren en te nemen voorzoren voor verantwoord gebruik als meststof. *KVIV Studiedag Mestproblematiek*, KVIV, Antwerp, Belgium.
[4] Garner H.V. (1957). Manures and Fertilizers. Great Britain. *Ministry of Agriculture, Fisheries and Food. Bulletin* 36, London, UK.
[5] Malley D.F., Yesmin L. and Eilers R.G. (2002). Determination of N, P, and salts in hog manure and manure-amended soils using near-infrared spectroscopy. *Soil Science Society of America Journa*l, 66, pp. 1677-1686.
[6] Milmier A., Lorimor J., Hurburgh C.Jr., Fulhage C., Hattey J. and Zhang H. (2000). Near-infrared sensing of manure nutrients. *Transactions of the ASAE*, 43, pp. 903-908.
[7] Reeves III J.B. and McCarty J.B. (2000). Determination of ammonium-N, moisture, total C and total N in dairy manures using a near infrared fibre-optic spectrometer. *Journal of Near Infrared Spectroscopy*, 8, pp. 151-161.
[8] Reyns P., Maertens K. and De Baerdemaeker J. (2001). On-line measurement of corn moisture content on a combine harvester using NIR technology. In: *Proceedings of the 3rd European Conference on Precision Agriculture (*Ed.) Grenier G. and Blackmore S., Montpellier, France, 2, pp. 827-832.
[9] Saeys W., Darius P. and Ramon H. (2004). Rapid on site analysis of hog manure using a visual and near-infrared diode array reflectance spectrometer. *Journal of Near Infrared Spectroscopy*, In Review
[10] Shenk J.S., Workman J.J.Jr. and Westerhaus M.O. (1992). Application of NIR Spectroscopy to Agricultural Products. In: *Handbook of Near-Infrared Analysis* (Ed.) Burns D.A. and Ciurczak E.W. Marcel Dekker Inc., New York, pp. 383-432.
[11] Williams P. (2003). *Near Infrared Technology – Getting the best out of light*. PDK Projects, Nanaimo, Canada