# Calibration of a near infrared-diode array system for biogas production potential in fresh forages

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## Introduction

Energy crop breeding is a key factor for the expansion of energy crop cultivation. For use as biogas, aside of other crop cultivation related traits, a high specific methane production potential is the most significant breeding aim. At present, the assessment of substrate-specific methane production capability is carried out in practice by batch tests as well as feeding-stuff analytical approaches. Both procedures are time and cost intensive and not appropriate for a fast genotype selection of fresh plant samples. Therefore, the development of a fast screening method to increase the efficiency of modern plant breeding programs is in high demand.

For quality analysis of agricultural products Near Infrared (NIR) Spectroscopy has proved itself as a cost-effective method. Furthermore, this technique has already been successfully applied by breeding companies for several years for on-line-estimation of important plant material properties on harvesting machines. In the sector of biogas production first attempts exist to use NIR spectroscopy for checking substrate quality, and process control of fermenters, as well as for the analysis of fermenting residues.<sup>1</sup>

At this time, one current project at the Institute of Crop and Soil Science of the JKI at Braunschweig aims at the development of NIRS-calibrations using fresh plant material under on-line conditions.

# Materials and methods

## **NIR-Instrumentation**

Habitually NIRS laboratory samples are normally measured in a dry and ground state. The specific features that affect spectral characteristics of freshly-chopped plant samples are the high water content (50–85%) which is also associated with high temperature sensitivity, as well as considerable differences in particle size, density, and homogeneity. Special technical requirements exist for such an on-line measuring system, including handling water, as well as in part additionally sugar rich samples, measurement geometry, shielding against too pronounced temperature shifts, and withdrawal of reference samples. Further technological needs are addressed to on-line measuring systems with regard to the extent of automation, performance, and process safety.

To free us from these technological requirements and the associated disturbances during the period of the development of calibrations, we have designed and developed an at-line test equipment (Figure 1).

Its configuration fulfils the most essential demands of a future on-line application on harvest machines and it is appropriate to be used as a peripheral station at an experimental field.

The chopped samples are placed in a 30L vessel that rotates beneath a detector head. This measuring head illuminates a  $2.5 \text{ cm}^2$  circle of the sample surface. The scan cycle of the reflectance signal consists of one sample rotation (total measuring time) and is repeated six times. For each measurement the sample was re-mixed. The measuring head is fibre-coupled to a Polytec 1720 NIR-spectrometer (Polytec, Waldbronn, Germany) covering the range from 850 to 1650 nm. After measurement, samples were stored at -20 °C until chemical analysis.



Figure 1. At-line measurement equipment.

#### Reference sampling and chemical analysis

For this study 59 maize samples, and 60 grass samples, as well as 16 whole crop small grain and 13 clover/grass samples were available. The estimation of the substrate specific methane production potential of fresh plant material was carried out in two ways. Firstly, by batch tests in 20 litre polyethylene bags, and secondly by equations for estimating the content of fermentable organic matter (FOM) as a parameter for predicting the methane production potential of maize and grass.<sup>2</sup> FOM is identical to the content of true digestible organic matter derived from strictly standardised digestibility trials in sheep. The average yield potential of methane yield per kg FOM of most crops were found by Weißbach to be about 420 litres and could be verified under practical conditions.

#### Mathematical procedure for calibration

The calibrations were carried out with the chemometric software package CWS (Calibrations Workshop, SensoLogic GmbH, Norderstedt). PLS-calibrations were calculated based on spectral and reference data from three data sets: maize, grass and a global set for all crop species. The following limits for different outlier types were used: *H* outlier (influence outlier) 3, *T* outlier (Student's test) 3, D outlier (Cook's statistic) 3 and S outlier (spectrum reconstruction error) 5. The model performance was characterised by *RMSECV* (standard error of cross validation),  $R^2$  (coefficient of determination of cross-validation) and *RPD* value (ratio of the standard deviation of the reference data for the validation samples  $SD_V$  and the SEP)<sup>3</sup> or  $RPD_{CV}$  value of cross validation, respectively.

# **Calibration results and discussion**

The calibration results of all three data sets for methane production potential that have been analysed by batch tests are unsatisfactory. The model performance is insufficient for practical purposes (cf. to Table 1).

Datat set		Ν	Mean	SEE	$R^2_{CAL}$	RMSECV*	$R^2_{CV}$	RPD <sub>CV</sub>
Batch- methane	global	155	335.82	33.80	0.44	35.61	0.37	1.27
	grass	60	351.94	33.86	0.47	38.39	0.32	1.21
	maize	59	324.32	22.69	0.41	23.60	0.35	1.25
FOM- methane	global	155	322.06	14.28	0.88	16.05	0.85	2.55
	grass	60	288.15	8.33	0.94	10.03	0.91	3.38
	maize	29	348.07	6.83	0.96	7.76	0.95	4.55

**Table 1.** Statistics of the calibration of comparable data sets of analyzed (batch test) and calculated methane production potential in undried forages.

Legend: \*litres methane/kg ODM; *SEE*: Standard error of estimate; *RMSECV*: Standard error of cross-validation;  $R^2$ : Multiple coefficient of determination;  $R^2_{CV}$ : Coefficient of determination of cross validation.

The errors of cross-validation ranging between 24 litres and 38 litres methane/kg organic dry matter (ODM) are too big in relation to the standard deviation of the data sets. For example the grass-set exhibits a standard deviation of 46 and the maize-set a standard deviation of 30 litres methane/kg ODM.

The calibration of the same data sets on base of dried and ground samples measured with the identical measure system leads to similar results (data not shown). This is surprising because, in contrast to fresh samples, a more favourable analytical signal-noise ratio could be expected.

For understanding the composition of the analytical error, fractionation of the substrate dry matter for its use in fermentation processes might be important.<sup>3</sup> The ODM can be subdivided into a fermentable portion (FOM) and a not usable portion. Depending on the conditions of the fermentation process in the FOM portion itself, the bacterial-transformed FOM and the unused FOM should be distinguished. Only the transformed FOM fraction, less the incorporated bacterial biomass, is available for biogas production.

This cascade-like fractionation reveals that at first the NIR-detection is only feasible for the identification of the FOM level. Nevertheless, a model prediction that includes the fermentation process presupposes high stability of process conditions. This does not seem to be given in the case of batch test conditioning. Our own analysis of batch test replications resulted in standard deviations between 8 and 10%.

By comparison, the possibility to generate usable calibrations for methane production potential was examined on basis of the FOM of each sample, calculated by prediction equations.<sup>4</sup>

The calibration results based on the three used data sets showed good model performance that seems to be applicable to freshly chopped plant samples.

#### Conclusion

Calibration experiments using NIR-spectra of freshly-chopped plant samples show that substratespecific methane production potential based on batch tests can only be predicted with poor model performance. The main reason is insufficient standardisation of these batch tests, which leads to poor reproducibility of results. The reference data for calibration involve an imposing error.

On the contrary, by use of the calculated substrate-specific methane production potential based on FOM, a model performance is achievable that seems to be applicable for practical screening purposes. Crop specific calibrations generate a better model performance than global calibrations.

In cases where higher accuracy is demanded, calibrations based on dried and ground samples are recommended.

## References

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