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Comparison of near infra-red spectroscopy, neutral detergent fibre assay and in-vitro organic matter digestibility assay for rapid determination of the biochemical methane potential of meadow grasses

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ABSTRACT

This paper investigates near infra-red spectroscopy (NIRS) as an indirect and rapid method to assess the biochemical methane potential (BMP) of meadow grasses. Additionally analytical methods usually associated with forage analysis, namely, the neutral detergent fibre assay (NDF), and the in-vitro organic matter digestibility assay (IVOMD), were also tested on the meadow grass samples and the applicability of the models in predicting the BMP was studied. Based on these, regression models were obtained using the partial least squares (PLS) method. Various data pre-treatments were also applied to improve the models. Compared to the models based on the NDF and IVOMD predictions of BMP, the model based on the NIRS prediction of BMP gave the best results. This model, with data pre-processed by the mean normalisation method, had an R^2 value of 0.69, a root mean square error of prediction (RMSEP) of 37.4 and a residual prediction deviation (RPD) of 1.75.

1. Introduction

Biogas is of relevance as a viable source of renewable energy as well as an option of using waste material such as activated sludge, manure and agricultural residue, amongst others. Biogas production using meadow grasses and low input cultivated grasses as feedstock is of growing interest because of the low energy and chemical input requirement for such grasses (Tilman et al., 2006), the abundance of grassland biomass and the possibility of transferring nutrients from grasslands to other locations whilst producing energy (Danmarks Naturfredningsforening, 2011). There has also been a decrease in the use of grasslands for animal husbandry especially in developed nations (Prochnow et al., 2009) due to improvements in breeding and production technology (Rösch et al., 2009). Murphy and Power (2009) argue in favour of using grassland biomass for the production of biogas in case of Ireland, especially, as they report that there will be a reduction in livestock in the future to meet emission requirements thus making grazing land available for bio-energy purposes. In Ireland, the BMP of grass per hectare was reported to be 4059 m^3 when 55% of the biogas was assumed to be methane, when compared to 2285 m^3 that can be obtained from wheat (Murphy and Power, 2009). Although maize has shown methane yields of 8778 m^3 per hectare in Austria (Amon et al., 2007) the input in terms of herbicides, irrigation and fossil fuel required for maize cultivation is higher than that required for grass (Tilman et al., 2006), and hence the use of meadow grasses as substrates in the production of biogas is of current interest.

As the number of commercial biogas plants grows, it will be increasingly important to assess the quality of the feed-stock in terms of their biochemical methane potential (BMP) which is a measure of the anaerobic biodegradability of a substance. This aspect is important, as the BMP of the feed-stock determines the feeding rate into a continuously run biogas reactor and could consequently determine the value of the feed-stocks. In addition, the design and operation of anaerobic digesters require data on the BMP of the substrate (Buffiere et al., 2006). The BMP assay, first proposed by Owen et al. (1979), is the most common method used to determine the total amount of methane obtainable from a substrate during anaerobic digestion. The recommendation by Owen et al. (1979) was to incubate the substrate for 30 days which would allow for virtually complete decomposition of biodegradable organics. However, in some cases, where the substrate is comparatively more recalcitrant, a digestion period that could be as long as 100 days may be required; an example can be seen in the study performed by Gunaseelan (2007). The time taken to degrade almost all the substrate will depend on various factors such as lignocellulosic content (Buffiere et al., 2006), substrate particle size (Mshandete et al., 2006), the activity level of the microbes in the inoculum (Neves et al., 2004) or the presence of inhibitors. Thus

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depending on the substrate sample, the inoculum, and the procedures used in the laboratory performing the tests, it takes a minimum of 30 days (Owen et al., 1979) to determine the BMP of a substrate. A faster way of arriving at the possible quantity of biogas and hence the quantity of methane that is obtainable from a particular substrate will be extremely useful.

Theoretically, if all the organic matter in a substrate is converted and if the substrate composition is known, the methane produced could be calculated using the Buswell's formula (Buswell and Mueller, 1952). This method takes into account the total amount of carbon, hydrogen and oxygen in the substrate and then using an empirical equation calculates the possible amount of methane produced. However, methane production is a complex biological process involving many factors and is lower than that predicted by the Buswell's formula. This is because some of the substrate is used to increase the bacterial biomass and whilst dealing with complex substrates like manure and agricultural residue not all the substrate can be biologically degraded. The quantity and the type of fibre present in the substrate will largely define the rate of substrate degradation which in turn determines the methane production that can be achieved using that particular substrate in a continuous reactor with a particular hydraulic retention time. The lignocellulosic content plays an important role in the amount of substrate that can be converted into methane in a given timeframe. Thus, the fibre content could feasibly be used as an indicator of the amount of biogas that can be produced. Many studies have tried to establish a connection between the chemical compositions of the substrates to their BMPs. Gunaseelan (2007) modelled the B_0 (the ultimate methane yield) values of fruit and vegetable wastes, Sorghum and Napier grass based on the carbohydrate, protein, acid detergent fibre (ADF), lignin and cellulose contents of the substrate. Eleazer et al. (1997) researched the connection between the cellulose, hemicellulose and lignin content of municipal refuse to its anaerobic biodegradability and found that not only did the amount of lignin in the substrate influence the BMP but the chemical composition of the lignin itself played an important role as well. There are two assays associated with forage analysis that assess the nutritional value of animal feeds based on the fibre contents and could be used to predict the BMP of substrates: the neutral detergent fibre assay (NDF) and the in-vitro organic matter digestibility assay (IVOMD). The IVOMD indicates the percentage of material that can be digested by rumen microbes. The NDF analysis indicates the total amount of fibre in a material, specifically the plant cell wall content which includes the cellulose, hemicellulose, lignin and mineral ash in a material (Richards et al., 2005). The IVOMD test takes about 4 days and the NDF assay takes around 2 days (including the time required for dry matter and ash contents analysis in both cases).

Another possibility that was investigated in this study was the use of near infrared spectroscopy (NIRS) where scanning of a prepared sample takes less than a few minutes. NIRS is a non-invasive rapid analytical technique that does not require chemicals (Kays et al., 2005). Many studies have focussed their attention on the application of NIRS in the process control of biogas production using process indicators such as volatile fatty acids (Hansson et al., 2002; Holm-Nielsen et al., 2008) and methanogen density (Zhang et al., 2002). Although NIRS is being vigorously researched in the field of anaerobic digestion, to our knowledge there has been just one other attempt by Lesteur et al. (in press) at using NIRS to predict the BMP of municipal solid wastes. Further research based on other substrates is necessary to make this technique applicable to real world situations.

The substrates used in this study were meadow grasses. The meadow grass samples were scanned with an NIR spectrometer and analysed using the NDF and IVOMD assays. The resulting data was analysed and models were developed based on the data.

2. Methods

2.1. Materials

The substrates tested in this study were meadow grasses; both mixed species and specific single species, collected from various locations in Denmark. These meadow grasses came from locations with different fertilisation strategies and harvesting regimes and were thus expected to provide a wide variation in their BMP. Predominant species in the mixed meadow grass samples were; *Phalaris arundinacea, Deschampia cespitosa, Holcus lanatus, Juncus effuses, Agrostis stolonifera, Glyceria fluitans* and *Alopecurus geniculatus*. Of these, *P. arundinacea, D. cespitosa, H. lanatus* and *J. effuses* were also collected individually and tested as single species. The grass samples were dried at 60 °C, cut to a size of 3–5 cm in length and stored in air tight bags to prevent sample degradation. The dry matter content of the grasses was 92% on average (standard deviation of 5.1) and the average volatile solids content expressed as the percentage of the dry matter content was 94% (standard deviation of 2.6). The grass samples were analysed for their BMP, IVOMD and NDF contents. Ninety five samples of meadow grasses were analysed for their BMPs, 106 for their IVOMD and 105 for their NDF and all the samples were scanned with an NIR spectrometer. The BMP of the meadow grasses was predicted using the NIR spectra, the IVOMD, the NDF, and the IVOMD and NDF combined.

2.2. Biochemical methane potential

The BMP was determined in triplicate based on the method described by Møller et al. (2004) and expressed in litres of methane produced per kilogram of volatile solids in the substrate (L/Kg VS). Fifteen grams of each of the dried and cut meadow grass samples were digested in individual batch reactors comprising of 1 litre glass bottles, each containing 400 g ± 5 g of inoculum. The inoculum was collected from the full scale biogas reactor located at the Foulum research centre, Tjele, Denmark, which is primarily fed a mixture consisting of 70% manure, 20% maize and 10% grass silage. The inoculum was de-gassed before use, by incubating it at a temperature of 35 °C ± 1 °C for a minimum of 15 days; this ensured that the biogas production of the inoculum itself was minimal. The batch reactors were then sealed and the headspace of each was flushed with 99.9% nitrogen gas to remove gaseous oxygen from the system. The batch reactors were then placed in an incubator that was maintained at a temperature of 35 °C ± 1 °C. The volume of the biogas produced in each of the batch reactors was measured regularly using an acidified water displacement method. The methane and carbon dioxide composition of the biogas was analysed every time the gas volume was measured by flushing a 22 mL sample bottle with 300 mL of biogas from one of each set of triplicates and then by analysing the gas sample on a Varian 3600 gas chromatograph equipped with a thermal conductivity detector. The temperatures of injection port, oven, filament and detector were 120 °C, 35 °C, 140 °C and 120 °C, respectively. The carrier gas was Helium with a flow rate of 30 mL min^{-1}. The experiment was carried out for a period of 90 days at the end of which the cumulative methane production per kilogram of volatile solids was determined.

2.3. In-vitro organic matter digestibility

The IVOMD assay was performed on dried and powdered samples according to the method proposed by Tilley and Terry (1963) using rumen fluid collected from fistulated cows (Foulum research centre, Tjele, Denmark) and the results were expressed in percentage of dry matter (%DM).

2.4. Neutral detergent fibre

The NDF assay was performed based on the method proposed by Van Soest and Wine (1967). The meadow grass samples were dried at 60 °C, powdered and analysed for NDF and the results were indicated in percentage of dry matter (%DM).

2.5. Near infra-red spectroscopy

The stored grass samples were dried once again at 60 °C for 24 hours and ground to a size of 0.8 mm or less using a Cyclotec sample mill (Foss, Denmark) and each of the samples was put into 20 ml glass bottles such that the sample filled up at least three fourths of the bottle. The surface of each of the bottles was wiped clean and then placed on a rotating powder sampler that was fitted onto the NIR spectrometer and the sample was scanned using a Bomem QFA Flex Fourier Transform spectrometer fitted with an InAs detector (Q-interline A/S, Copenhagen, Denmark). The spectroscopy was completed in the reflectance mode.

2.6. Data analysis

BMP prediction models were created using The Unscrambler v9.8 software (CAMO Software A/S, Oslo, Norway). All the models were based on partial least squares regression for single parameter prediction – PLS1 models. The following models were made; NIRS prediction of BMP, IVOMD prediction of BMP, NDF prediction of BMP and a model using both IVOMD and NDF to predict the BMP. Two other models - NIRS prediction of IVOMD and NIRS prediction of NDF were built to compare with similar NIRS based models proposed by previous studies thus supporting the NIRS method being used in this study.

In the case of the NIR spectra some amount of pre-processing was required to ensure that a good model was obtained. The entire range of the spectra measured by the instrument was 77 cm^{-1} to 15,522 cm^{-1}, removing the noise regions outside the near infrared range reduced the spectral range to a band between 4000 cm^{-1} to 12,000 cm^{-1}, this range of spectra will be referred to as raw data. Models were built on raw data as well as pre-processed data and the models with the best prediction statistics have been presented in the results section. A variety of data pre-processing algorithms available within The Unscrambler v9.8 software were applied to the raw data to improve the models. Various normalisation methods like area normalisation, unit vector normalisation, range normalisation, maximum normalisation, mean normalisation, and peak normalisation were used. Derivative pre-processing methods such as Savitzky-Golay derivative and the Norris-Gap derivative were also used. Other than these, multiplicative scatter correction, de-trending, standard normal variate, linear baseline correction and baseline offset were also applied.

The R^2 values and the root mean square error of prediction (RMSEP) values were used as measures of good modelling, where R^2 is the co-efficient of determination and is defined as the proportion of variance explained by the regression model and is useful as a measure to the success of prediction (Nagelkerke, 1991). The root mean square error of prediction (RMSEP), expresses the average error to be expected with future predictions (Esbensen et al., 2002). Thus a high R^2 and a low RMSEP is desirable in a model. Another measure of the models is the residual prediction deviation (RPD) which is the ratio of standard deviation to the standard error of prediction. This is particularly useful in comparing the prediction abilities between alternative models (Lomborg et al., 2009). It is recommended that the RPD should be higher than 10, although when the standard deviation of the data set is not high the RPD cannot reach 10. In such cases where the sample set is quite uniform and the standard deviation of the data set is low the RPD should indicate that the RMSEP is appreciably lower than the standard deviation (Williams and Norris, 1987). The validations of the models were performed using leave one out cross validation as the data set was small.

3. Results and discussion

The results obtained from the BMP assays, the IVOMD and the NDF analyses including their ranges and their arithmetic means are presented in Table 1. The frequency analyses of the data sets are presented in the Supplementary material. The models with the best validation statistics are summarised in Table 2.

The prediction accuracy for each of the models can also be seen in Table 2 along with the standard deviations of the data sets that were used to calculate the RPD values. The corresponding actual versus predicted plots are presented in Fig. 1a–f.

The application of NIRS to predict the BMP of the grass samples resulted in the best prediction statistics with R^2 of 0.69 and an RPD of 1.75 (Fig. 1a) when compared to the prediction of BMP by NDF or IVOMD (Fig. 1b and c). The pre-processing of the NIRS spectral data improved the models only slightly. Lesteur et al. (in press) conducted a similar study using NIRS to predict BMP based on municipal solid wastes (MSW) along with separated fractions of the MSW and obtained a model with an RPD of 2.36.

The NIRS was also used to predict the IVOMD and NDF (Fig. 1e and f). The R^2 and RMSEP values seen whilst modelling the NIRS spectra to the NDF and the IVOMD values of the grasses are similar to those observed in previous studies. Andrés et al. (2005) used near infra-red spectroscopy to predict in-vitro gas production parameters using herbage from natural meadows as substrates. The IVOMD gas production model had an R^2 value of 0.801 and an RPD value of 2.797 which are similar to the results of this study.

Table 1
Biochemical methane potentials (BMP), in-vitro organic matter digestibility (IVOMD) and neutral detergent fibre (NDF) contents of the meadow grass samples.

	BMP L/Kg VS	IVOMD % DM	NDF % DM
Minimum	51.0	31.8	40.4
Maximum	405.6	73.3	70.7
Mean	287.5	56.3	59.0
Median	307.2	55.9	59.1
Standard deviation	65.7	8.5	6.2

Table 2
Validation statistics of the models.

Model	Data pre-processing	Number of samples (n)	Number of principle components	RMSEP	R^2	Standard deviation of data set (SD)	RPD (SD/RMSEP)
NIRS → BMP	Mean normalisation	95	13	37.4	0.69	65.7	1.75
NDF → BMP	Raw data	90	1	49.5	0.27	57.4	1.16
IVOMD → BMP	Raw data	91	1	44.1	0.41	57.3	1.30
IVOMD and NDF → BMP	Raw data	91	1	45.2	0.38	57.3	1.27
NIRS → IVOMD	Baseline offset	106	9	3.5	0.83	8.5	2.42
NIRS → NDF	Baseline offset	105	9	2.5	0.84	6.2	2.48

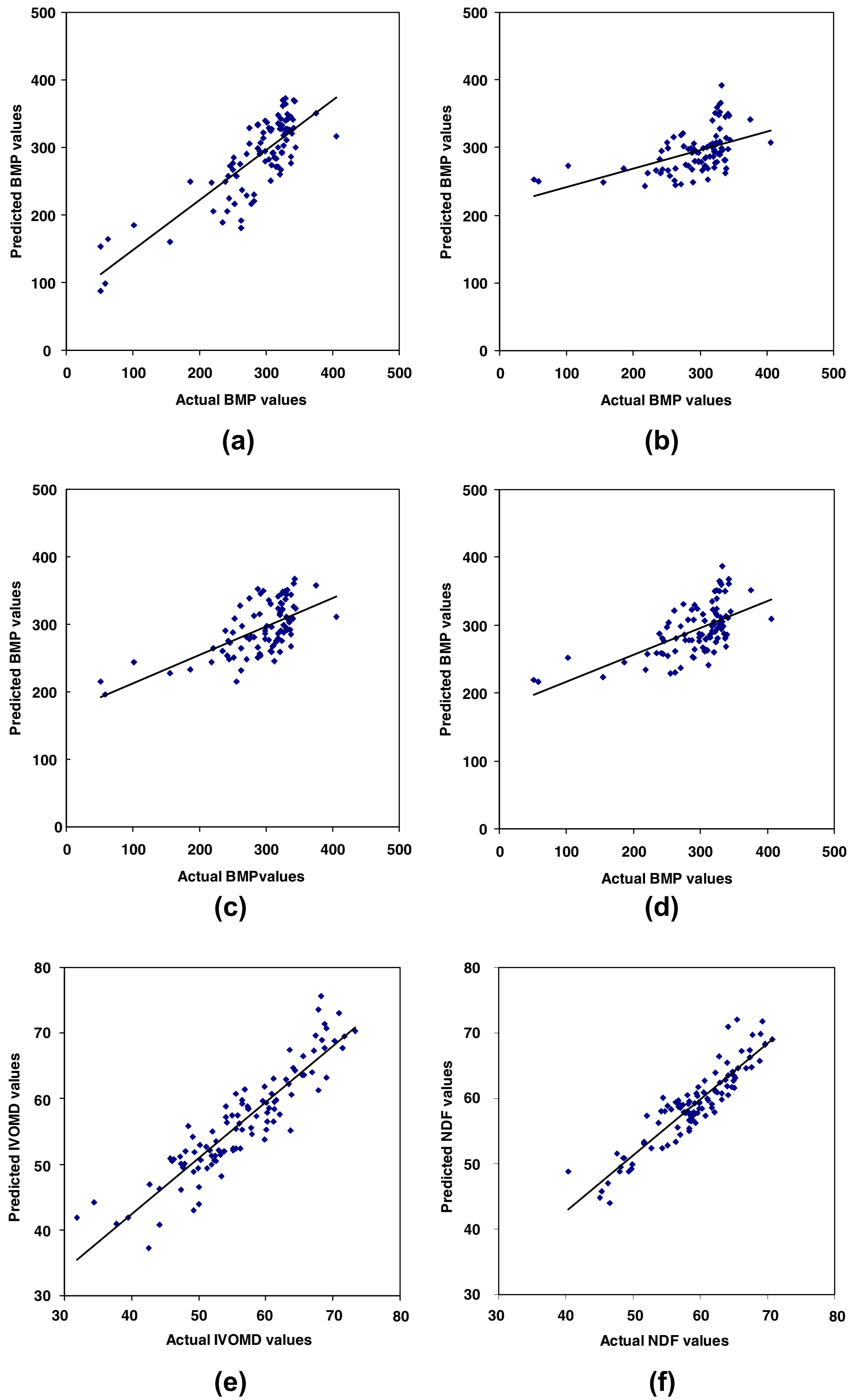

Fig. 1. Validation plots of the models, (a) NIRS prediction of BMP, (b) NDF prediction of BMP, (c) IVOMD prediction of BMP, (d) IVOMD and NDF prediction of BMP, (e) NIRS prediction of IVOMD, and (f) NIRS prediction of NDF.

The prediction statistics of BMP using IVOMD and NDF were poor compared to those obtained by using NIRS. Between them, the IVOMD determination of BMP shows better calibration statistics than the NDF determination of BMP (Fig. 1c and b). This could be due to the inclusion of the biological factor in IVOMD. The NDF represents the easily degradable material in a sample, and in a BMP assay at least a large portion of the more difficult to degrade material is also mineralised, which indicates that the NDF prediction of BMP is less accurate. Combining the IVOMD and the NDF to predict the BMP, as seen in Fig. 1d did not substantially improve the prediction.

A few possible ways of improving the BMP prediction models based on NIRS could be identified from the results of this study. The BMP data set was quite homogeneous, with a few low values, a BMP data set with a wide range of values might have resulted in better prediction statistics, as can be seen in the work of Lesteur et al. (in press). Another issue could be the lack of sample homogeneity: the sample used to obtain the spectra is powdered, which increases homogeneity, whereas the samples that were introduced into the BMP batch reactors were dried grass samples that were 3–5 cm in length. The connection between NIRS and BMP could possibly be improved by powdering and homogenising the substrate before the BMP assay, this will ensure a homogenous sample and hence a more accurate BMP estimation. Although this might help in improving the model, it might not reflect real world conditions. The ideal condition would be where the NIRS could be used scan the substrate as it goes into a reactor and predict the BMP, but this would require a different sampling set up for the NIRS. An example of direct sampling can be seen in the work of Jacobi et al. (2011) where the NIRS set up was modified and used to monitor the quality of feedstock to a Biogas production plant directly.

There are numerous examples of studies to model BMP based on chemical and biological parameters of the substrates. Using ADL, ADF, lignin content, total soluble carbohydrate, nitrogen and ash contents of fruits and vegetables solid wastes, Sorghum and Napier grass models with high R^2 values were built (Gunaseelan, 2007). These results show a good co-relation to the BMP; however, they still require relatively more time and require the aid of various chemicals for analyses when compared to NIRS. With respect to the present study, compared to NDF and IVOMD and the two combined together, the NIRS has the best prediction capability, and although the R^2 can be improved, this study proves that there is a possibility of using NIRS in the prediction of BMP, and provide an estimate of the BMP of a substrate. In comparison to the BMP assay which takes as minimum of 30 days to provide an estimate of the amount of methane that a substrate can produce, the NIRS takes very little time (2 minutes per sample in this case). The current study has modelled the BMP of Danish meadow grasses using their near infra-red spectra. If the same concept is extended to various other substrates, NIRS could be used in the management of Biogas plants where assessing the methane potential of the feedstock would influence the substrate loading rate, and a quick estimate of the BMP of a substrate would prove to be a useful tool.

4. Conclusions

This study investigated NIRS, IVOMD and NDF as rapid BMP assessment tools and in conclusion, NIRS seems to be the best option with an R^2 value of 0.69, an RMSEP of 37.4 and an RPD of 1.75. The IVOMD and NDF, both individually and combined were not as good as the NIRS in predicting the BMP.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.05.049.

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