CHARACTERIZATION OF KEY PARAMETERS FOR BIOTECHNOLOGICAL LIGNOCELLULOSE CONVERSION ASSESSED BY FT-NIR SPECTROSCOPY. PART II: QUANTITATIVE ANALYSIS BY PARTIAL LEAST SQUARES REGRESSION

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Wheat straw (*Triticum aestivum* L.) and oat straw (*Avena sativa* L.) were chemically pretreated at different severities with the purpose of delignification, which in turn leads to a better accessibility of plant cell wall polysaccharides for further biotechnological conversion. Key parameters of these samples, i.e. weight loss, residual lignin content, and hydrolysable sugars serving as precursors for biofuel production were monitored by wet-chemistry analyses. Fourier transform near infrared (FT-NIR) spectra were correlated to these data by means of partial least-squares (PLS) regression. Weight loss (4.0 – 33.5%) of the wheat straw could be predicted (RMSEP = 3.5%, R^2_{test} = 0.75) from the entire FT-NIR spectra (10000 – 4000 cm^{-1}). Residual lignin content (7.9 – 20.7%, RMSEP = 0.9%, R^2_{test} = 0.94) and amount of reducing sugars based on pretreated wheat straw (128 – 1000 mg g^{-1}, RMSEP = 83 mg g^{-1}, R^2_{test} = 0.89) were powerfully evaluated between 6900 and 5510 cm^{-1}, a spectral region where polysaccharides and lignin absorb. All these parameters could be equally predicted with even higher accuracy from pre-treated oat straw samples. Furthermore, some important parameters for anaerobic conversion of wheat straw to biogas – biogas production, total solids, and volatile solids content – could be estimated.

Keywords: Wheat and oat straw pre-treatment; Fourier transform near-infrared spectroscopy (FT-NIR); Biogas potential test; Anaerobic fermentation; Multivariate data analysis; Partial least-squares (PLS) regression

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INTRODUCTION

Wheat and oat straw are lignocellulosic materials primarily containing 30-35% cellulose, 20-25% hemicelluloses, and 17-20% lignin. The complex structure of the lignocellulose complex is considered as the major obstacle for polysaccharides utilization in fermentation processes (Himmel et al. 2007; Chandra et al. 2007). Pre-treatment is required to open up the lignocellulose structure and to increase the accessibility to microbial enzymes, which hydrolyze the carbohydrate constituents to fermentable sugars. These sugars are regarded as precursor substances for biofuels or building blocks for

chemical syntheses. Lignin removal, hemicellulose depolymerisation and solubilization, swelling of the fibre structure, decrease of cellulose crystallinity, etc., may be results of the pre-treatment (Kumar et al. 2009).

To assess the properties of pretreated lignocellulosic materials, a number of wet-laboratory methods have been proposed and standardized (Energy Efficiency & Renewable Energy, US Department of Energy 2009). These mostly destructive methods are costly, time-consuming, and tiresome. Alternatively, Fourier transform near-infrared spectroscopy (FT-NIR), which is a simple, powerful, and sensitive non-destructive tool can provide the information of chemical and physical properties of lignocelluloses and other food and agricultural products (Ghosh and Rodgers 2001; Shenk et al. 2001; Siesler et al. 2002, Ozaki et al. 2007). NIR absorption bands are derived from the overtones and combinations of, i.e. , C-H, N-H, O-H, and C=O vibrations. By this spectroscopic means, not only the chemical structure modification but also the physical and morphological changes of organic substances can be characterised. Combined with multivariate data analyses, a number of studies on quantitative analyses of lignocelluloses and cereals composition by means of NIR spectroscopy have been reported (Kong et al. 2005; Fackler et al. 2007a,b). Partial least-square (PLS) regression is an efficient mathematical tool revealing the quantitative information of the spectral data. Recently, many distinct NIR bands of lignocellulose materials have been assigned (Tsuchikawa and Siesler 2003a; Tsuchikawa and Siesler 2003b; Tsuchikawa et al. 2005; Watanabe et al. 2006; Mitsui et al. 2008), providing more descriptive explanatory details for understanding the multivariate regression models.

In the first part of the study (Krongtaew et al. 2010), FT-NIR spectra of 80 pretreated wheat straw samples and 53 pretreated oat straw samples were discussed qualitatively using multivariate tools of data analysis (principal component analysis). In this second part of the study the same FT-NIR spectra were used to assess the lignin content, reducing sugar, and weight loss of the pretreated samples analysed quantitatively using partial least-squares (PLS) regression. This work has shown the powerful assessment of key parameters for biotechnological conversion of biomass, particularly biogas yield, based on characteristics of pretreated lignocelluloses which have not been mentioned before.

EXPERIMENTAL

Materials

Wheat straw (*Triticum aestivum* L.) and oat straw (*Avena sativa* L.) were obtained from Wirtschaftsbetriebe Herbert Rauch Höpfner, Vienna, Austria from the 2005 harvest. Wheat straw contained 63% polysaccharides, 21.5% lignin, 11.4% extractives, and 4.2% ash, while oat straw contained 51% polysaccharides, 19.6% lignin, 20.5% extractives, and 8.9% ash. These data were from wet-chemistry analyses. All chemicals for pre-treatment and analytical assays were p.a. grade and purchased from Sigma-Aldrich (www.sigmaaldrich.com). Digested sludge for anaerobic digestion was kindly contributed from the wastewater treatment plant in Schwechat, Austria, and cattle manure

was kindly provided by the Teufel farm in Zipf and the Gradinger farm in Mühlheim/Inn, Upper Austria.

Methods

Pre-treatment of straw

Wheat and oat straw were chopped to 1-cm length. 20 g dry straw was treated in 200 mL solution (solid-to-liquid ratio of 1:10) at different concentrations of acid, acid/H_2O_2, alkali, and alkali/H_2O_2. The initial pH of pre-treatment in acidic (pH 2.5 to 4.5) and alkaline (pH 9 to 12) conditions was adjusted by concentrated sulphuric acid (> 95%) and 1 $molL^{-1}$ sodium hydroxide, respectively. Some pre-treatments were carried out in the presence of different amounts of hydrogen peroxide (2% to 10% w/w based on dry straw). The pre-treatment was carried out for 4 h in a water bath at different temperatures varied from room temperature (25°C) to 90°C. After pre-treatment, straw was washed with 1 L distilled water and dried for at least 24 h at 50°C for analyses. This moderate temperature was used in order to prevent chemical modifications of pretreated straw during drying, which might influence FT-NIR spectra.

Enzyme hydrolysis of treated straw samples

Prior to hydrolysis, crude Viscozym L (www.novozymes.com) was purified by Econo-Pac 10DG column (www.bio-rad.com), using water as eluent to eliminate low molecular weight compounds like sugars and salts. Afterwards, 100 mg straw (dry basis at 50°C) was hydrolysed in enzyme solution containing 1 mL purified Viscozym L and 9 mL 30 $mmolL^{-1}$ sodium-acetate buffer pH 4.0. The enzyme hydrolysis was performed at 40°C for 48 h, and subsequently 1 mL of supernatant was centrifuged and analysed for reducing sugar content.

Inoculum seed preparation and biogas potential test

To get the equal quality of inoculum for the biogas potential test, inoculum seed was prepared in 2-L semi-continuously stirred tank reactor (CSTR) system as illustrated in Fig. 1. To the 2-L final volume, 800 mL raw sludge and 200 g cow manure, 200 mL nutrient solution (0.03 gL^{-1} NH_4Cl, 0.2 gL^{-1} K_2HPO_4, 0.05 gL^{-1} $MgSO_4 \cdot 7H_2O$, and 0.01 gL^{-1} yeast extract), and tap water were added. The initial pH was adjusted to 7.2 and maintained during the digestion by adding 1 $molL^{-1}$ NaOH. The temperature for anaerobic digestion was controlled at 37°C, and 2 gVS $L^{-1}d^{-1}$ of organic loading rate of wheat straw (2 mm particles) was fed daily. Biogas generated was measured by compact automated displacement gas metering system (Angelidaki et al. 1992). The digestion was carried out until the system reached the steady state condition as indicated by 1) constant biogas production, 2) constant substance concentration (volatile fatty acid and alkalinity), and 3) constant destruction of straw (total solid and volatile solid removals). Then, the sludge mixture was ready to use as inoculum seed for the biogas potential test in batch fermenter.

For the batch biogas potential test, 1 g pretreated straw (oven dry basis) and 150 mL inoculum seed from the CSTR system was added into a 500-mL Erlenmeyer flask. The anaerobic digestion started at 37°C after flushing the head space with nitrogen gas. The mixture was shaken twice daily. Accumulative biogas generation was recorded by a

liquid displacement method using acidified water (12 molL^{-1} hydrochloric acid for pH 2-3) to prevent solubilization of CO_2 (Wilkie et al. 2004). Total solids and volatile solids removals of sludge mixture were determined after the gas generation ceased. Biogas yield referred to accumulative biogas volume divided by straw dry weight (mL biogas g^{-1} straw).

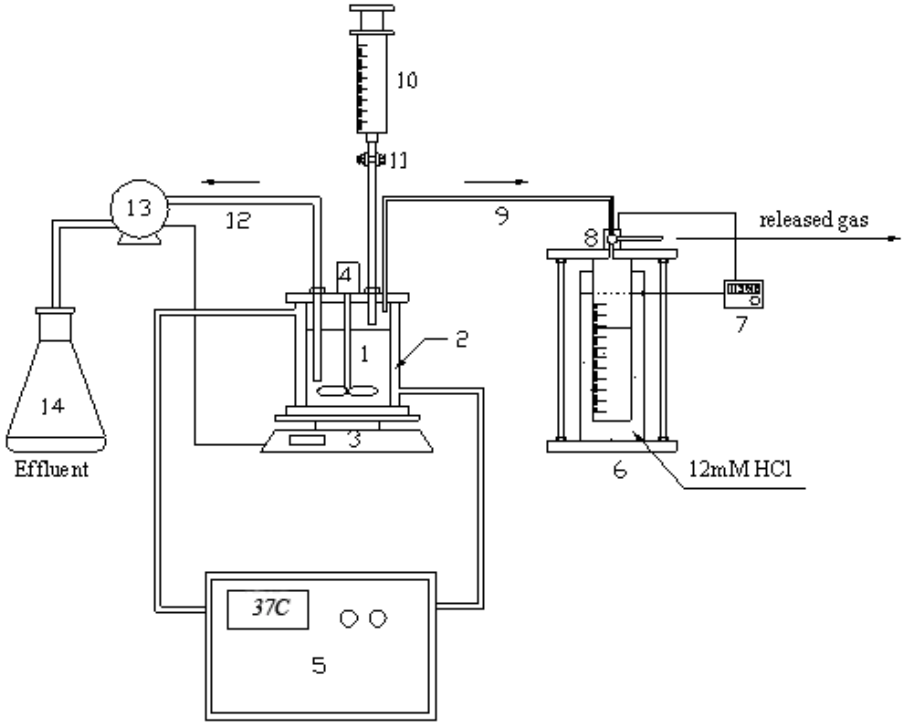

Fig. 1. Semi-continuous anaerobic digestion for inoculum seed preparation: (1) 3-liter continuously stirred tank reactor (CSTR), (2) water coil, (3) weight balance, (4) motor for stirrer, (5) temperature controlled water bath, (6) continuous gas collecting column, (7) counting device, (8) gas release valve, (9) gastight tube, (10) syringe for feeding substrate, (11) airtight valve, (12) effluent tube, (13) liquid pump, and (14) effluent flask

Analyses

Weight loss of pretreated straw was determined after drying at 50°C for 24 h. Reducing sugars in supernatant after enzyme hydrolysis were determined by a modified dinitrosalicylic (DNS) acid method. Reducing sugar yield (mg sugar g^{-1} pretreated straw) was calculated based on glucose equivalent from the calibration curve. As the method does not differentiate between reducing end groups of pentoses and hexoses, the pentoses was overestimated (factor = 1.2). Dry straw samples were then milled to 80 μm particles using the ultra-centrifugal mill (Retsch ZM 1000, Germany) for analyses of extractives, Klason lignin, acid-soluble lignin, and FT-NIR spectroscopy. Total solids and volatile solids content were also monitored for pretreated samples stored at the same relative humidity and temperature. The procedures were reported elsewhere (Krongtaew et al. 2010).

Fourier transform near-infrared (FT-NIR) spectroscopy

FT-NIR spectroscopy of milled samples (80 µm particles) was carried out in terms of apparent absorbance, log [1/Reflectance], by FT-NIR spectrometer using a fiber-optic probe (Equinox 55, Bruker Optics Inc., Germany). Spectroscopy was performed with 8 cm^{-1} resolution, 100 scans (60 seconds), and wave numbers ranging from 10000 to 4000 cm^{-1}. The average spectra of 4 replicates were calculated.

Partial least-squares (PLS) regression models between FT-NIR spectra and the key parameters from straw pre-treatment, namely total lignin content, amount of reducing sugars, and weight loss, were set up by OPUS QUANT 2 version 6.0 (Bruker, Germany). In some cases, spectra were differentiated to second derivatives (17 smoothing points, 2nd order polynomial fit, using the Savitzky and Golay algorithm (1964)), or minimum/maximum normalized in the respective spectral range (Tables 1 and 2). From 80 wheat straw samples and 53 oat straw samples, half of the samples were chosen randomly for the calibration and the remaining samples were used as the test set to validate the models. The validity of models was indicated by the determination coefficient of test samples (R^2_{test}) and root mean square error of prediction (RMSEP). The ranges of wavenumber for setting up the PLS models of lignin content, reducing sugar, and weight loss were chosen using OPUS QUANT 2 Optimization. The other ranges (7500/ 7200 – 5500 cm^{-1}) covered 1st overtones of O-H and C-H stretching bands of water, lignin, and polysaccharides (Krongtaew et al. 2010). The PLS models of total solid, volatile solids and biogas yield, the crucial parameters for anaerobic fermentation, were calculated from the alternate sample set (42 wheat straw samples). Due to the smaller number of samples, leave-one-out cross validation was performed.

RESULTS AND DISCUSSION

FT-NIR spectra

In Fig. 2, pre-processed FT-NIR spectra are shown in the spectral region used for the regression models. As illustrated in Fig. 2(B) and 2(C), 2nd derivative FT-NIR spectra of straw samples after different severities of physico-chemical pre-treatment show significant changes in the range between 7200 and 5500 cm^{-1}. This spectral region is attributed to CH stretch 1st overtone vibration of the aromatic lignin structure, which is overlapped with CH stretch 1st overtone vibration band of acetyl groups (CH_3) of hemicelluloses, mainly xylan in straw (Shenk et al. 2001; Fackler et al. 2007b; Tsuchikawa et al. 2005). Moreover, modification of FT-NIR spectra ranging from 7200 to 6000 cm^{-1}, attributed to alteration of OH stretch 1st overtone of amorphous, semi-crystalline, and crystalline cellulose (Tsuchikawa and Siesler 2003a; Tsuchikawa and Siesler 2003b; Watanabe et al. 2006) , were also observed after physico-chemical pre-treatment. Qualitative changes in FT-NIR spectra after lignin degradation as well as degradation or deacetylation of polysaccharides were described in detail in the preceding article (Krongtaew et al. 2010).

During the physico-chemical pre-treatment, lignin and hemicelluloses were solubilized, and the crystalline structure of the substrate was substantially changed. These changes were reflected in the FT-NIR absorbance. PLS regression models of FT-NIR

spectra between 6900 and 5510 cm^{-1} were set up for predicting total residual lignin content and the amount of hydrolysable reducing sugars of pretreated straw samples (Table 1). This spectral region includes a lignin band near 5980 cm^{-1} and xylan bands near 5800, 5960, 5990 cm^{-1} (Krongtaew et al. 2010) as well as information on cellulose crystallinity (Fig. 2(B)). A further PLS model was calculated from the entire FT-NIR spectra ranging from 10000 to 4000 cm^{-1} to estimate the weight loss after pre-treatment (Table 1). Weight loss leads to loss of NIR absorption of C-H, O-H, N-H, and C=O vibration bands of organic substances (Fig. 2(A)).

Table 1. Parameters and Errors from Partial Least-Squares (PLS) Regression Models of Total Residual Lignin, Reducing Sugar and Weight Loss of Delignified Wheat and Oat Straw

Models	Substrate	Component range	No. of samples/ No. of test samples	Spectral region (cm^{-1})	Spectral pre-processing
Total residual lignin (%w/w)	Wheat straw Oat straw	7.9 - 20.7 8.3 - 18.5	80 / 40 53 / 27	6900- 5510 6900- 5510	2nd derivative 2nd derivative
Reducing sugars (mg g^{-1}- straw)	Wheat straw Oat straw	128 - 1000 131 -812	80 / 40 53 / 27	6900- 5510 6900- 5510	2nd derivative 2nd derivative
Weight loss (%)	Wheat straw Oat straw	4.0 - 33.5 5.0 - 44.0	80 / 40 53 / 27	10000-4000 10000-4000	No processing No processing

Models	Substrate	[1]R^2_{cal}	[2]RMSEE	[3]R^2_{test}	[4]RMSEP	No. of PLS Factors
Total residual lignin (%w/w)	Wheat straw Oat straw	0.95 0.99	0.9% 0.5%	0.94 0.96	0.9% 0.8%	3 1
Reducing sugars (mg g^{-1}- straw)	Wheat straw Oat straw	0.94 0.96	45 mg g^{-1} 54 mg g^{-1}	0.89 0.93	83 mg g^{-1} 64 mg g^{-1}	4 1
Weight loss (%)	Wheat straw Oat straw	0.85 0.96	2.9% 2.6%	0.75 0.91	3.5% 3.4%	4 4

[1] Coefficient of determination of the calibration model
[2] Root-mean-square error of estimation
[3] Coefficient of determination of the test model
[4] Root-mean-square error of prediction for future analysis

Lignin content, amount of reducing sugars and the weight loss of oat straw of the pretreated samples were accurately evaluated from FT-NIR spectra by means of PLS regression (Table 1 and Fig. 3). Good correlations between NIR-predicted and measured values were obtained for calibration and test sets, as indicated by high values of the determination coefficients (R^2_{cal} and R^2_{test}) and low root-mean-square error of estimation and prediction (RMSEE and RMSEP). These model parameters and the low number of factors required to obtain this high correlation are good indications for the robustness and applicability of the regression models.

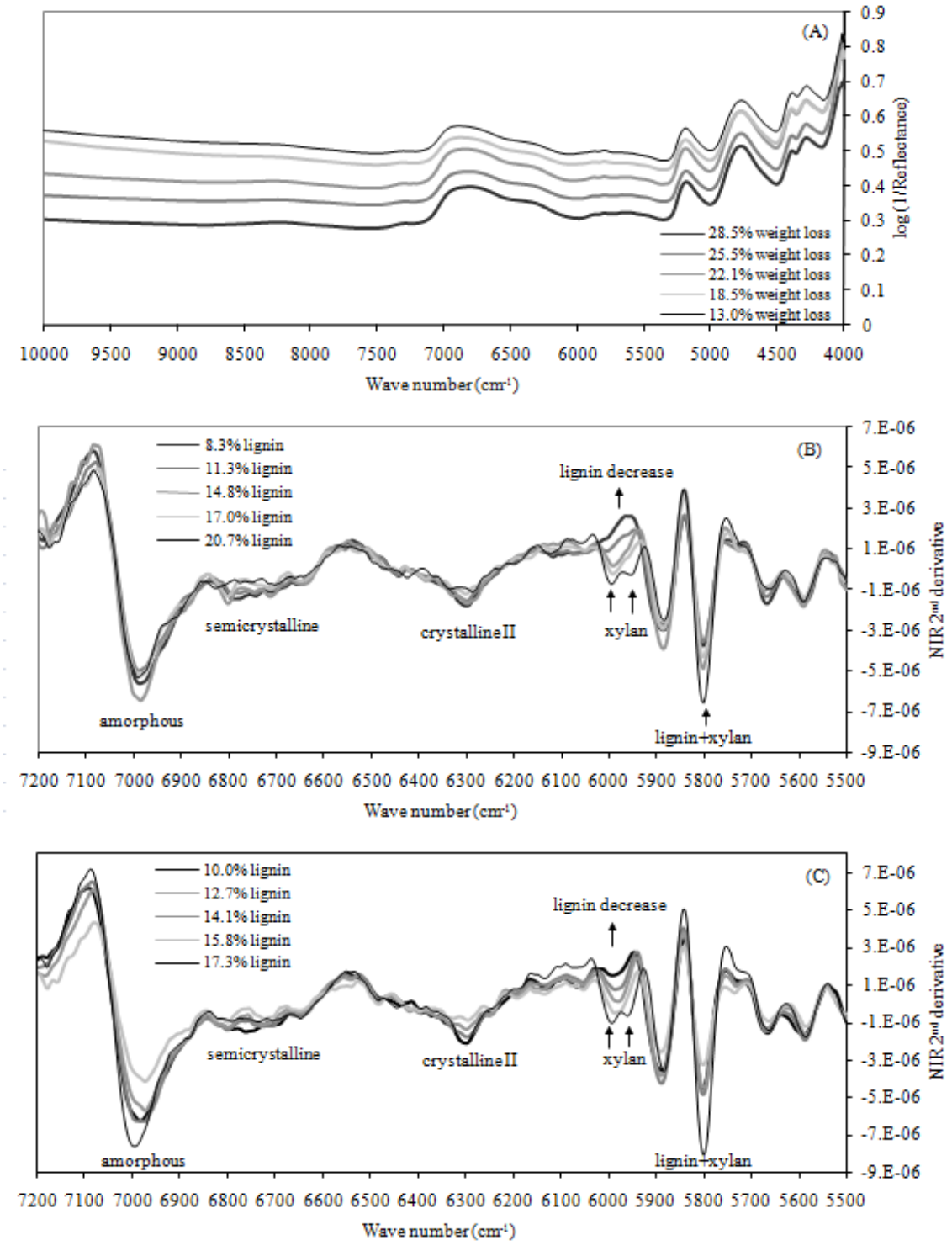

Fig. 2. FT-NIR spectra of wheat straw (A), and second derivative of FT-NIR spectra of pretreated wheat (B) and oat straw (C) samples containing different residual lignin contents

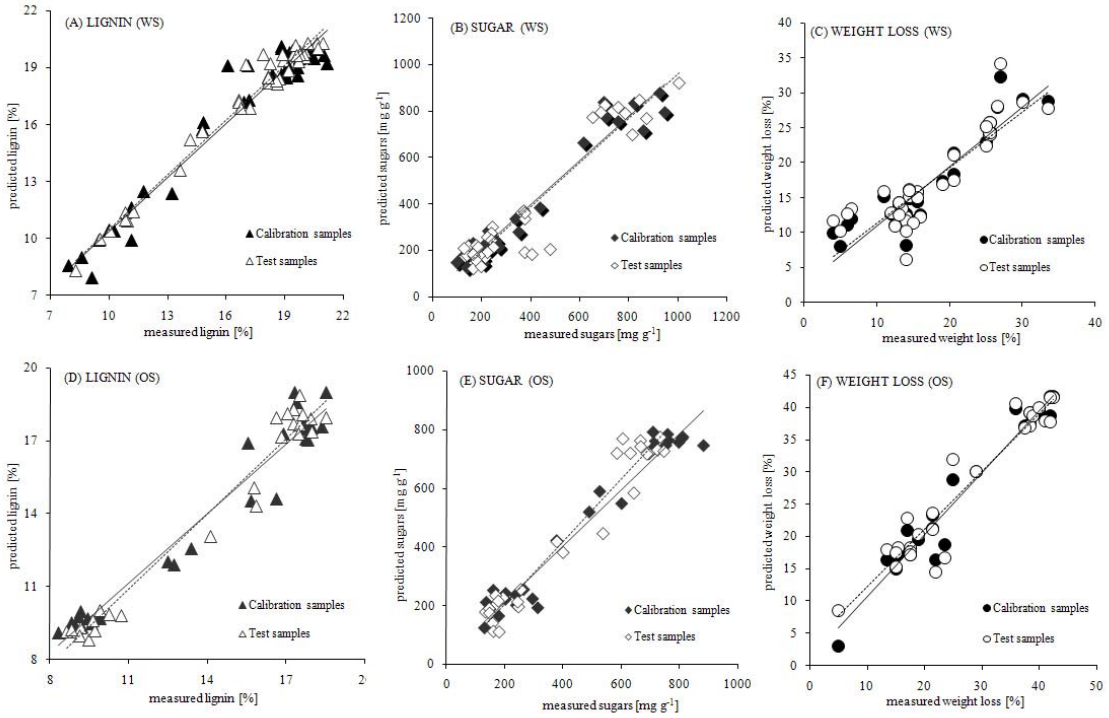

Fig. 3. NIR-predicted and measured values of the PLS regression models of total residual lignin content (A, D), reducing sugars (B, E) and weight loss (C, F) of calibration and test samples of pretreated wheat (WS) and oat straw (OS) samples

The re-sampling method was applied for the models to ensure the reliability of all the PLS models, as they were limited by a smaller number of observations when dividing the sample into two halves for calibration and test sets (Yu 2003). Ranges of R^2_{test}, RMSEP, and number of PLS factors of ten different calibration and test sets demonstrated consistent and reliable results, as shown in Table 2. The results showed the robustness and validity of the models for predicting total residual lignin, reducing sugar, and weight loss of physic-chemically pretreated wheat and oat straw samples.

Table 2. Re-Sampled Models of Ten Different Sub-Sample Sets for PLS Models of Total Residual Lignin, Reducing Sugar and Weight Loss

Models	Substrate	Spectral region (cm^{-1})	Spectral pre-processing	[1]R^2_{test}	[2]RMSEP	No. of PLS Factors
Total residual lignin (%w/w)	Wheat straw	6900 – 5510	2nd derivative	0.92-0.95	0.8-1.0 %	3
	Oat straw	6900 – 5510	2nd derivative	0.95-0.98	0.6-0.8 %	2-3
Reducing sugars (mg g^{-1} straw)	Wheat straw	6900 – 5510	2nd derivative	0.82-0.92	53-96 mg g^{-1}	3-4
	Oat straw	6900 – 5510	2nd derivative	0.92-0.96	45-65 mg g^{-1}	1-3
Weight loss (%)	Wheat straw	10000 – 4000	No processing	0.65-0.73	3.3-4.1 %	3-4
	Oat straw	10000 – 4000	No processing	0.84-0.93	2.4-3.9 %	4-5

[1] Coefficient of determination of the test model
[2] Root-mean-square error of prediction for future analysis

Assessment of Wheat Straw-to-Biogas Conversion by FT-NIR

To optimize the PLS models for total solid content, volatile solid content, and biogas yield, some outliers were excluded from the 42 original samples, thus the numbers of samples were different from those shown in Table 3. Although R^2_{cv} of those cross-validated PLS models for total solid, volatile solid, and biogas yield were not as high as for those described above (Table 3 and Fig. 5), the current models deliver a good estimation of these parameters. Furthermore, the much higher R^2_{cal}, the low number of PLS factors, and the low bias of these models indicate the high potential of the FT-NIR method, also to analyse these parameters.

Table 3. PLS Regression Models from FT-NIR Spectral Data of Pretreated Wheat Straw

Parameters	Total solids	Volatile solids	Biogas
Wave number [cm^{-1}]	7200 - 5500	7200 - 5500	7500 - 5500
Component range	93.6-99.7 %	94.5-99.2 %	219-387 mL g^{-1} straw
Spectral processing	Second derivative	Second derivative	Min-Max normalization
No. of samples	32	38	30
No. of PLS factors	5	5	5
Calibration			
R^2_{cal}	0.91	0.91	0.77
RMSEE[1]	0.7 %	0.3 %	21 mL g^{-1} straw
Cross validation			
R^2_{cv}	0.80	0.75	0.65
RMSECV[2]	0.9 %	0.5 %	23 mL g^{-1} straw
Bias	-0.05	0.03	0.73

[1] Root mean square error of estimation
[2] Root mean square error of cross validation

An acceptable prediction of biogas yield was obtained (R^2_{cv} = 0.65) from pretreated straw spectra when the consistency of inoculum seed quality and digestion condition were controlled. Thus, the analytical technology using the implementation of FT-NIR spectroscopy in the combination of mathematically spectral data processing could be applied to the entire system of biomass-to-bioenergy processes.

Biogas yield was well correlated to the amount of fermentable sugars available as carbon source for anaerobic digestion and also to the lignin content of the pretreated straw (Table 4). It was additionally observed that the yield of anaerobic digestion was influenced by the particle size of wheat straw (80 μm and 2 mm) (Fig. 4). Pretreated straw with smaller particles (80 μm) gave higher biogas yields compared with larger particles (2 mm), while lignin content and the amount of reducing sugars of the same samples from wet-chemistry analyses were at the same levels. The reason is most likely due to better accessibility of microbial enzymes toward the smaller particle size of straw. Moreover, the coefficient of correlation (R) between lignin and biogas as well as reducing sugar and biogas (Fig. 4(A), 4(B)) were higher when determined among the same sets of particle size compared with those from all samples (Table 4). The PLS model, however, included all particle sizes.

Table 4. Correlation Matrix of Key Parameters for Biomass-to-Biogas Conversion of Wheat Straw

	Lignin	Reducing sugars	Weight loss	Total solid	Volatile solid	Biogas
Lignin	R=1.0	R=-0.8	R=-0.9	R=-0.2	R=-0.4	R=-0.6
Reducing sugars	-	R=1.0	R=0.8	R=0.1	R=0.2	R=0.6
Weight loss	-	-	R=1.0	R=0.0	R=0.4	R=0.1
Total solid	-	-	-	R=1.0	R=-0.4	R=0.3
Volatile solid	-	-	-	-	R=1.0	R=-0.2
Biogas	-	-	-	-	-	R=1.0

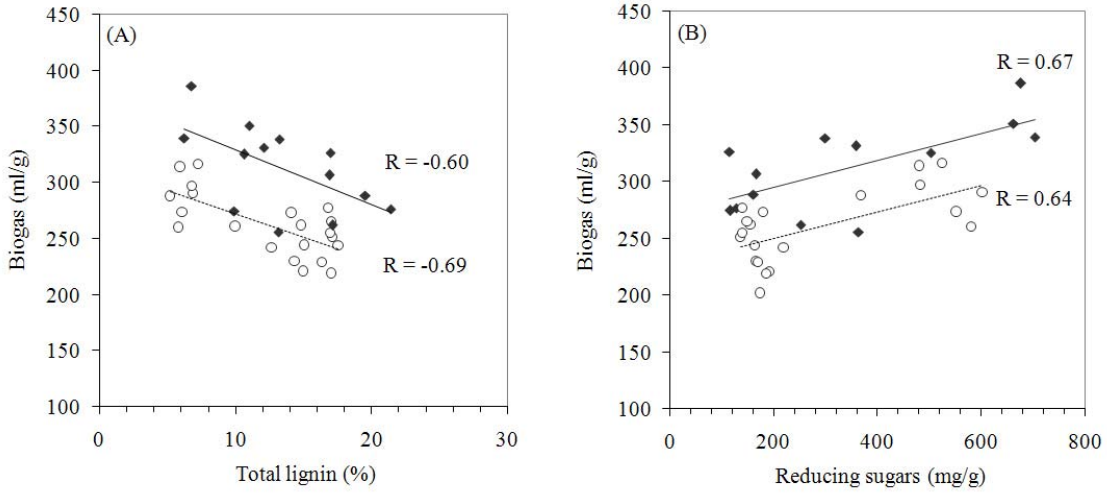

Fig. 4. Correlation between total lignin and biogas (A), and enzymatically released reducing sugars and biogas (B), where ♦ and ○ represent data from 80-µm and 2-mm straw samples, respectively

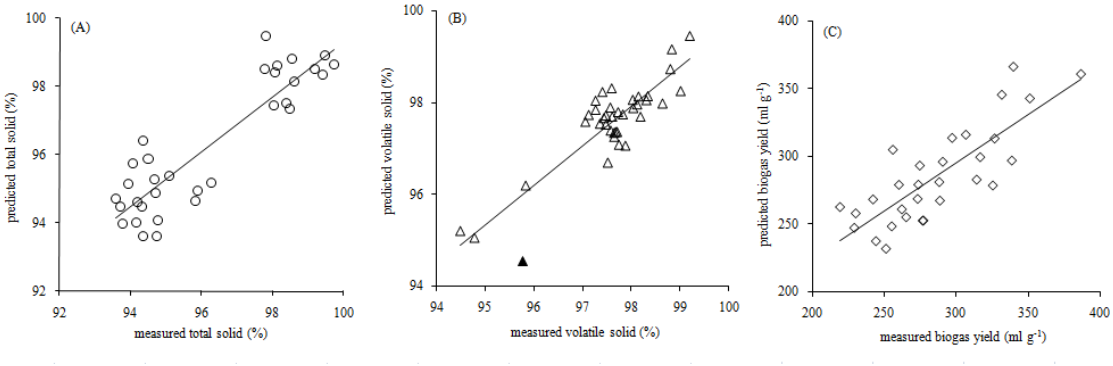

Fig. 5. NIR-predicted and measured values of (A) total solid, (B) volatile solids, and (C) biogas yield from the cross validation of PLS regression models of pretreated wheat straw. Black solid indicates the outlier.

A recent study demonstrated the prediction of biological methane potential based on a number of parameters - carbohydrates, proteins, lipids, acetate, and propionate concentration of a digested sludge mixture (Kaparaju et al. 2009). Some scientific work also demonstrated the potential application of NIR for predicting volatile solids, total solids, chemical oxygen demand (COD), and several volatile fatty acids (VFA) of digested sludge from the biogas pilot plant; the models relied mainly on different process constraints and were designed as analytical techniques for online-process control (Holm-Nielsen et al. 2006, 2007). The present study, in contrast, demonstrates the assessment of biogas yield based on chemical structure and composition of the substrate by FT-NIR spectroscopy. Biogas yield is based on the stoichiometric conversion of organic material to biogas as shown in equation (1). Volatile organic compounds in lignocelluloses are theoretically converted to methane (CH_4) and carbon dioxide (CO_2), which are collectively called biogas (Buswell and Mueller 1952). In most methanogenic fermentations, the methane yield lies close to the theoretical maximum of 3 moles of methane per mole of glucose, as calculated from the Buswell equation (1) (Ahring and Westermann 2007). Thus, the theoretical methane yield of untreated wheat straw is approximately 10.5 mmol CH_4 and 10.5 mmol CO_2 per g straw, as wheat straw contains 63% polysaccharides (glucose equivalence). These values were calculated to 235.2 mL CH_4 and 235.2 mL CO_2, as one mole of an ideal gas at STP occupies 22.4 L.

$$C_aH_bO_c + \left(a - \frac{b}{4} - \frac{c}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4}\right)CO_2 \qquad (1)$$

However, due to the complex structure, which is rather hard to attack, lignocellulose leads to yields lower than this theoretical maximum. The presence of organic recalcitrant compounds in straw, e.g. lignin and crystalline cellulose, influences the yield of anaerobic digestion. These properties of the substrate are reflected in the FT-NIR spectra and can serve for the prediction of the potential biogas yield. For instance, it has been reported that some parts of the crystalline structure of cellulose are loosened and

converted to semi-crystalline structures after physico-chemical pre-treatment (Krongtaew et al. 2010). This could lead to higher susceptibility of cellulose and provided higher biomass utilization efficiency.

PLS Loading Spectra

PLS loading spectra contain the descriptive information of FT-NIR absorption regions that influence the PLS regression models. As shown in Fig. 6(A), the first loading factor (PLS1, 2nd derivative pre-processing) of lignin content represents 82% of the data variance, and PLS1 of reducing sugar represents 75% of the data variance. The major fraction of the spectral variance influencing these models is found between 7200 and 5500 cm^{-1}, where lignin, xylan, and acetyl groups of polysaccharides absorb. As shown by highest negative amplitudes near 5980 cm^{-1}, the spectral position is attributed to the C-H stretch 1st overtone of aromatic rings, and the prediction of the lignin content is mainly based on this band. But a high lignin content is also correlated with a low xylan content, as indicated by the negative PLS loading vectors of lignin+polysaccharides bands near 5800 cm^{-1}, the overlapping band of C-H stretch 1st overtone of lignin and acetyl groups (Lg+Xy, Fig. 6A), and the broad band of amorphous polysaccharides near 7000 cm^{-1}. Bands assigned to crystalline II (6280 cm^{-1}) and semi-crystalline (6722 cm^{-1}) cellulose, however, exhibit positive loading vectors, indicating high cellulose content of the samples with low lignin content and *vice versa*. It is known that lignin degradation of lignocellulosic substrates leads to better accessible and enzymatically hydrolysable polysaccharides and thus to a higher yield of reducing sugars. In fact, these two parameters showed a high negative correlation of R = -0.8 in the data set analysed here (Table 4). This correlation was also found in the first loading spectrum of the model for reducing sugars, which is very much alike that of the lignin model but shows the opposite algebraic sign.

The correlation matrix (Table 4) shows the low correlation of total solids with all other parameters determined. Consequently, PLS-loading spectra (Fig. 6(B)) were not directly connected to the content of the straw constituents. Nevertheless, most of the data variance (62%) was explained by the second and third PLS loading factor (PLS2 and PLS3, 2nd derivative pre-processing) and the regression model showed good predictable results for total solid content (Table 3 and Fig. 5(A)).

For the PLS loading spectra (2nd derivative pre-processing) of volatile solids (Fig. 6(C)), which is the content of combustible organic materials, PLS1 and PLS3 contributed mostly to the PLS model. In the PLS1 loading spectrum, lignin and xylan overlapping band (Lg+Xy) showed the highest influence on the model. Amorphous polysaccharides (Am) near 7000 cm^{-1} and lignin-polysaccharide overlapping band near 5800 cm^{-1} (Lg+Ps) also appeared. A high content of volatile solids was predicted from pretreated straw sample, which contained low lignin and xylan (Lg+Xy) content as well as lignin and polysaccharide overlapping (Lg+Ps) but high amount of crystalline (Cry II), semi-crystalline (Sc), and amorphous (Am) cellulose, indicating the extraction of inorganic compounds during the pre-treatment.

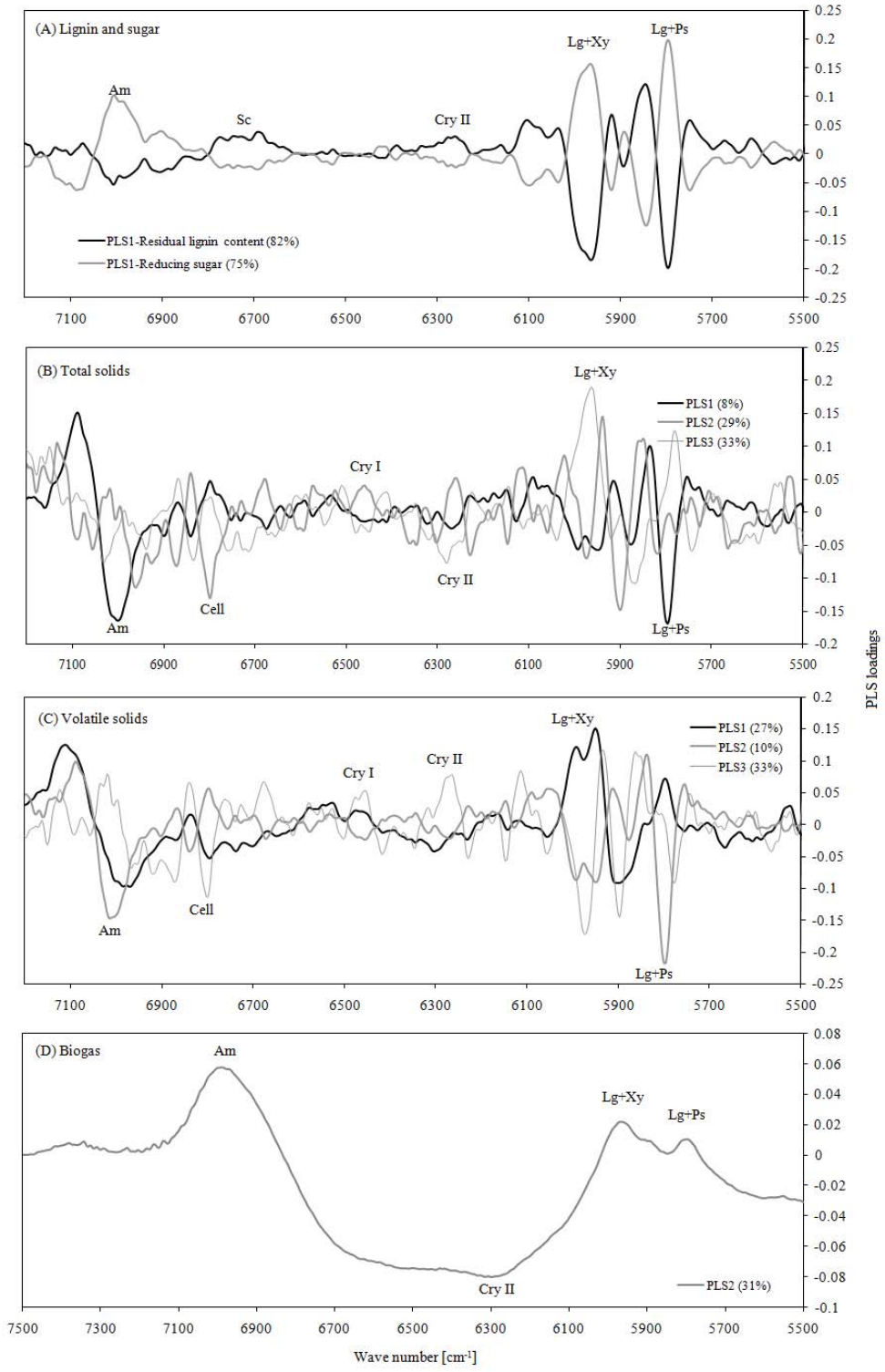

Fig. 6. PLS loading spectra of partial least-squares regression models: (A) lignin content and amount of reducing sugar, (B) total solids, (C) volatile solids, and (D) biogas yield.

The first PLS factor of PLS regression models (minimum/maximum normalization pre-processing) of biogas accounted for only 1% of the data variance; therefore the second PLS factors is discussed here (Fig. 6(D)). PLS2 represented 31% of the data variance and showed the importance of amorphous (Am) polysaccharides for a high biogas yield. Crystalline cellulose (Cry II), however, had an adverse effect, as indicated by a negative loading vector of PLS2 in the respective region.

In summary, it can be seen from the PLS loading spectra that lignin, xylan, and both amorphous and crystalline moieties of cellulose were representative onto the PLS model for evaluating biogas yield based on straw chemical structure after different severities of various physico-chemical pre-treatment methods. Thus, biogas potential of physico-chemically pretreated straw can be predicted based on the chemical characteristics and composition by FT-NIR, assisted by multivariate data analysis.

CONCLUSIONS

Fourier transform near-infrared (FT-NIR) spectroscopy combined with multivariate data analyses has potential as an analytical tool for not only qualitative characterization of lignocellulose after different pre-treatment methods through the vibration bands of FT-NIR spectra (Krongtaew et al. 2010), but also quantitative assessment for key parameters of both pre-treatment and biomass-to-bioenergy production. Total residual lignin content, enzymatically released reducing sugars, total solids (TS), volatile solids (VS), and biogas yield can be analysed. This approach can be considered as an alternative process analytical technology (PAT) for assessment of biomass utilization and biomass-to-biogas production offering a powerful and low-budget technique for rapidly growing and bulk volume industries.

Furthermore, the descriptive information in the loading vectors confirmed the importance of delignification for successful enzymatic and microbial conversion of the structural polysaccharides as well as the adverse effect of high cellulose crystallinity.

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