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Line monitoring by near-infrared chemometric technique for potential ethanol production from hydrothermally treated *Eucalyptus globulus*

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ABSTRACT

This study reports a method that combines near-infrared (NIR) measurements with multivariate analysis to predict the saccharification efficiency of hydrothermally pretreated *Eucalyptus globulus* during ethanol conversion. Optimization of the NIR data with or without spectral treatment determined the best calibration model in the region 10000–4000 cm^{-1} of the original spectra, with an RMSEP of 2.08% and R_p^2 of 0.99. By investigating the regression coefficient to understand the key regions and chemical components, for original and multiplicative scatter correction (MSC)-treated spectra, the water absorption and higher wavenumber regions were important. For the second derivative spectra, the regression model was constructed based on the CH overtone vibrations (6050–5500 cm^{-1}). The regression coefficient demonstrated that the removal of hemicellulose resulted in higher lignin content, which might affect the biomass properties in terms of water absorption and enhanced enzymatic hydrolysis evaluated by dinitrosalicylic acid (DNS) method. For a higher throughput system, aqueous sample analysis was performed using an immersion probe equipped with an InGaAs detector, which generated an acceptable calibration model having RMSEP of 4.25% and R_p^2 of 0.94. These results show the great potential of NIR spectroscopy for achieving fast, accurate, and nondestructive analysis, and its highly adaptability for maintaining an ethanol bioconversion system.TGS, triglycine sulfate

1. Introduction

The chemical composition and ultrastructure of plant cell walls greatly influence their enzymatic digestibility during ethanol conversion, a process which is of increasing interest due to energy shortages from the excessive consumption of non-renewable fuels. A line-monitoring system, defined as a rapid and reproducible analytical method, is needed to support a stable conversion process; current monitoring uses conventional wet chemical analyses that involve many steps and employ additional reagents, increasing the environmental load, expense, and time requirements. Near-infrared spectroscopy is a fast, non-destructive, and powerful method for the characterization of organic materials. It provides information about the structure, chemical microenvironment of the constituent biomolecules, and their functional group distributions. Its combination with multivariate analysis, known as chemometrics, enables the simultaneous quantification of chemical components as well as assessment of the physical properties of plant materials [1,2].

This quantitative method has been applied to biomass samples, as initially reported by Sanderson et al. [3], who demonstrated the establishment of a calibration model between NIR spectra acquired in the reflectance mode and chemical composition, such as individual carbohydrate, lignin, and ash contents. Later studies investigated calibration models for systems employing various NIR spectral pretreatments [4,5] alone or in combination with other multivariate analysis methods such as PCA [6] and SIMCA [7], or the interpretation of regression vectors or loading factors [8]. For chemically pretreated rice straw and *Erianthus*, we previously demonstrated a chemometric approach to the saccharification ratio, which is an important index for assessing pretreatment methods [9,10].

Abbreviations: NIR, near-infrared; MSC, multiplicative scatter correction; FPA, filter paper activity; DNS, dinitrosalicylic acid; TGS, triglycine sulfate; PLS, partial least squares; RMSEP, root-mean-square error of prediction; RPD, ratio of performance to deviation; RMSEC, root-mean-square error of calibration.

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Table 1
Statistical summary of the saccharification data for the calibration and prediction sets.

	Calibration set					Prediction set				
	Samples	Max. (%)	Min. (%)	Mean (%)	S.D. (%)	Samples	Max. (%)	Min. (%)	Mean (%)	S.D. (%)
Saccharification ratio (%)	54	62.36	4.95	27.48	16.82	18	61.49	5.80	29.42	17.76

All of the NIR spectra conducive to the acceptable calibration model were measured from residues that had strongly NIR-light absorbing and scattering properties, with a few sample preparation steps such as drying or molding. Therefore, this NIR measurement was limited to "at-line monitoring" in terms of a manufacturing control system for ethanol conversion. On the other hand, liquid sample measurement would enable direct screening without dilution or extensive sample preparation, which promises higher throughput analysis corresponding to "in-line monitoring". Needless to say, the use of NIR spectroscopy for aqueous analysis has been difficult because the signals of interest are obscured by strong and broad vibrational bands due to water [11]. However, this challenge has been overcome by tweaking measurement conditions or spectral instrumentation. In a successful example, Rodriguez-Saona et al. [12] constructed a calibration model using different sampling devices, ultimately finding that the transmittance mode achieved the rapid analysis of polysaccharides in fruit juices by NIR spectroscopy. Cozzolino et al. [13] also created an acceptable calibration model for the phenolic compounds in red wine fermentation by NIR spectroscopy in the transmittance mode. Liebmann et al. [14,15] applied a fiber optic probe to successfully predict components such as glucose and ethanol in liquid samples during fermentation.

The present study initially reports the development of a high-throughput analysis using NIR spectroscopy to predict the digestibility of hydrothermally treated *Eucalyptus globulus* from hydrolyzed residues. The spectral pretreatment and region were optimized to construct a better regression model. Next, to more fully understand the relationships between the NIR spectra and enzymatic hydrolysis toward efficient ethanol conversion, a regression coefficient was calculated and interpreted in terms of chemical and structural components. Finally, for a higher throughput system for practical bioprocess monitoring, we tried to construct a

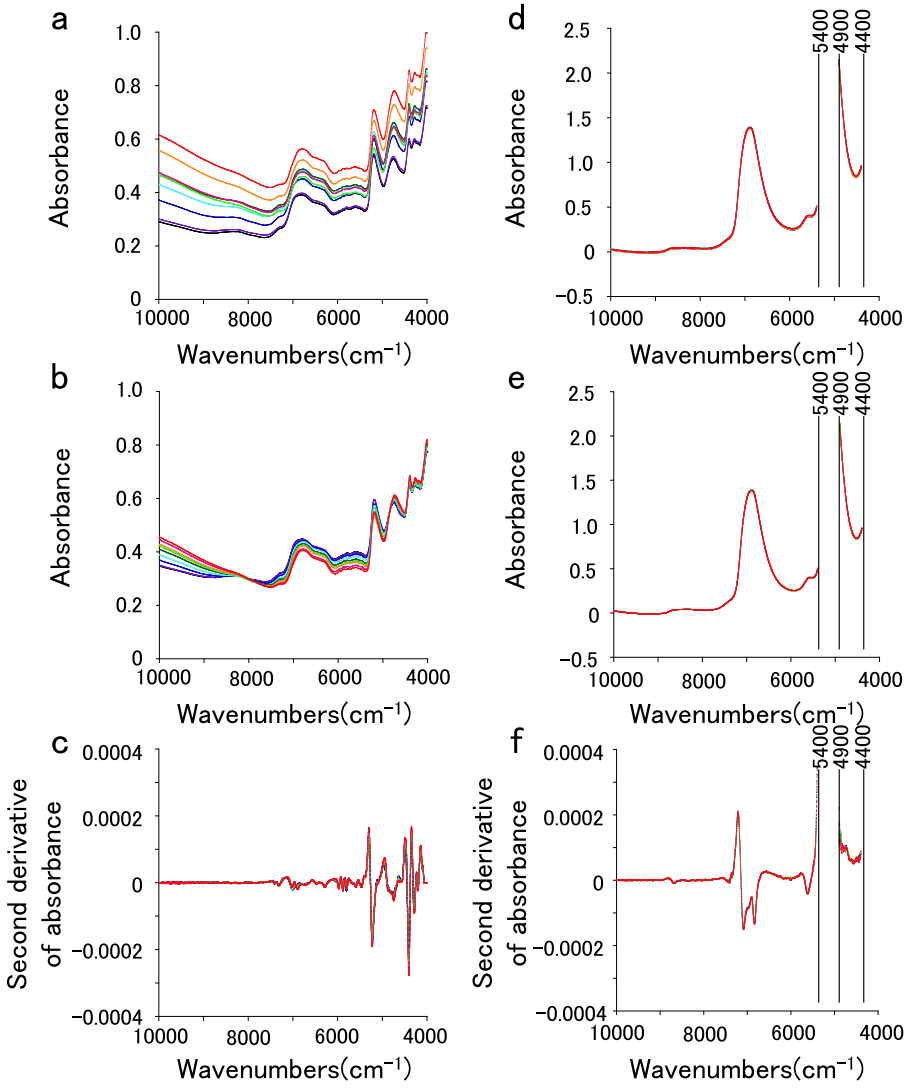

Fig. 1. NIR spectra obtained from solid samples by sphere diffuse reflectance (a–c) and from liquid samples by immersion probe (d–f) are shown. (a) and (d) are original, (b) and (d) are MSC-treated, and (e) and (f) are second-derivative spectra. The nine spectra were obtained from hydrothermal treatment at 190 °C for various times (1–64 min).