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Applicability of near-infrared spectroscopy for process monitoring in bioethanol production

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ABSTRACT

The applicability of near-infrared (NIR) spectroscopy to bioethanol production is investigated. The NIR technique can provide assistance for rapid process monitoring, because organic compounds absorb radiation in the wavelength range 1100–2300 nm. For quantification of a sample's chemical composition, a calibration model is required that relates the measured spectral NIR absorbances to concentrations. For calibration, the concentrations in g/l are determined by the analytical reference method high performance liquid chromatography (HPLC). The calibration models are built and validated for moisture, protein, and starch in the feedstock material, and for glucose, ethanol, glycerol, lactic acid, acetic acid, maltose, fructose, and arabinose in the processed broths. These broths are prepared in laboratory experiments: The ground cereal samples are fermented to alcoholic broths ('mash'), which are divided into an ethanol fraction and the residual fraction 'stillage' by distillation. The NIR technology together with chemometrics proved itself beneficial for fast monitoring of the current state of the bioethanol process, primarily for higher concentrated substances (>1 g/l).

1. Introduction

Near-infrared (NIR) spectroscopy has become an accepted analytical technique in food [1], agricultural [2], and pharmaceutical [3] industry and even entered medical applications, e.g., blood analysis for diabetes patients [4]. Despite its success in many applications, NIR spectroscopy is still new to many industrial areas and often neglected due to the difficult – if not impossible – interpretation of NIR spectra by the naked eye. Differences in samples may cause only hardly detectable differences in the NIR spectra, because of broad and overlapping NIR bands [5]. However, adequate mathematical and statistical methods have been developed in chemometrics to access the information hidden in the spectra, for instance by multivariate calibration with reference data [6].

The NIR technique offers many advantages in industrial environments. To name a few:

- Online NIR analytics are easily implemented using long distance fiber optic probes.
- A single NIR spectrum allows the determination of a variety of different substances.
- The analytical signal is recorded within milliseconds, and can be evaluated automatically using predefined calibration models [2].

In this work, we investigate the applicability of NIR spectroscopy to media present in bioethanol production. Therefore, laboratory experiments are performed, and NIR calibration models for different substances are built with reference data from HPLC (high performance liquid chromatography). All models are optimized, and thoroughly validated to estimate the confidence intervals of errors for future applications.

2. Materials and methods

2.1. Feedstock

In bioethanol production from cereals, the most important components of the incoming grain are starch, protein, and moisture. Starch is the source of fermentable sugars for yeast, thus it is directly affecting the yield of ethanol. The protein content is determining the quality of the valuable by-product of bioethanol distillation, an animal feed called DDGS (distillers' dried grains with solubles). Only high quality incoming grain with a certain protein level can meet the DDGS protein level guaranteed to the customers. The critical moisture level is about 15%, higher values can cause problems during feedstock storage as well as mechanical problems in the grain mill. A quick monitoring of the incoming grain by NIR spectroscopy is an asset for bioethanol plants, as low quality feedstock can be rejected in due time [7].

The feedstock materials considered in this work comprise wheat, rye, corn, barley, and triticale flours. In total, 15 differ-

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ent flour samples were available for feedstock analysis with NIR spectroscopy. The applied NIR method is described in Section 2.4. Reference data were obtained by wet chemistry analyses as described in Section 2.3.

For quantification of moisture, the 15 cereal samples were not used, as storage conditions changed the water content before NIR spectra could be measured. Alternatively, an online dataset containing NIR spectra of 100 wheat samples with wet chemical reference data for the moisture content [8] was obtained from ftp://ftp.clarkson.edu/pub/hopkepk/chemdata/.

2.2. Bioethanol fermentation

In laboratory-scale batch systems, 19 bioethanol fermentations were performed using different feedstock cereals: eight experiments with wheat, eight experiments with rye, and three experiments with corn. The mashes (cereal and water) were standardized to 195 g/l starch. Provided a complete fermentation with 100% yield, the alcoholic mashes then contained 100 g/l ethanol.

Starch-containing feedstock requires a two-stage enzymatic degradation of starch to glucose, to supply yeast with a digestible starting compound for alcohol synthesis [7]: In the first stage, called 'liquefaction', starch is split into shorter oligosaccharides with varying chain length. The used enzyme is α-amylase that randomly breaks the α-1,4 glucosidic linkages in starch. In the second stage, called 'saccharification', the enzyme glucoamylase acts upon the chain ends of the available oligosaccharides and releases single glucose molecules. For the experimental starch degradation, the mashes were liquefied with α-amylase for 1 h at 90 °C, and then cooled to 60 °C for a 3-h saccharification process with glucoamylase. In all experiments, the same type of α-amylase and glucoamylase enzymes were used. In wheat and rye experiments, however, supplementary enzymes were added at different stages of the mashing process to improve the processing properties (e.g., viscosity) of the mash. In total, six different enzymes were used for additional treatment.

The preprocessed mashes were then fermented with dry bakers' yeast at 30 °C, pH 4.5, and at anaerobic conditions over a holding period of 64 h. In the three experiments with corn, 22 samples were taken during the fermentations, and at their ends. Samples from wheat and rye experiments, in total 16, were only drawn at the end of the fermentations.

For distillation, the alcoholic mashes from wheat and rye (but not corn) were transferred into a rotary vacuum evaporator, and were separated into a high-in-ethanol vapor fraction and the so-called stillage as distillation residue. After distillation, in total 12 stillage samples were withdrawn, 4 from wheat stillages and 8 from rye stillages.

2.3. Sample preparation and analysis

The solid cereal samples available for feedstock analysis were ground to a median particle size smaller than 0.3 mm, and stored in containers. The starch content was determined after enzymatic conversion of starch to glucose, and subsequent glucose analysis by high performance liquid chromatography (HPLC, see below). The used concentration is, in fact, 'starch calculated as glucose' in g/l. The protein content in the feedstock was determined by the ICC standard method No. 105/2, 'Determination of crude protein in cereals and cereal products for food and feed'. 'The organic matter of the sample is oxidized with concentrated sulfuric acid in the presence of a catalyst: the product of the reaction $(NH_4)_2SO_4$ is treated by alkali; free ammonia is distilled and titrated.' [9] Crude protein results from the total nitrogen content in the sample multiplied by the factor 6.25.

The liquid samples were taken during fermentation, from the final alcoholic mashes, and from the stillages that remained after evaporation of ethanol. Every sample was centrifuged for 10 min at 5000 rpm to separate a clear liquid from the solids (e.g., yeast cells, fibers). The clear samples were mixed with sodium azide to limit bacterial growth, and were stored at 4 °C until further preparation. At this stage, 38 clear mash samples and 12 clear stillage samples were collected and subjected to HPLC analysis (BIO-RAD AMINEX ion exclusion HPX-87H column, $T = 60$ °C, flow rate: 0.5 ml/min; mobile phase: 2.5 mM H_2SO_4, RI detector) to determine the concentrations of ethanol, glucose, fructose, arabinose, maltose, and the fermentation by-products glycerol, lactic acid, and acetic acid.

The clear samples were diluted with water, divided into subsamples, and then mixed with different, known amounts of glucose, ethanol, glycerol, lactic acid, or acetic acid. Eventually, the samples were measured by NIR spectroscopy (see Section 2.4). On the one hand, this procedure ensured that the calibration samples cover a large concentration range. On the other hand, the number of calibration samples increased to 166 mash samples and 75 stillage samples. A higher number of diverse samples promotes a reasonable calibration and validation of the mathematical models that relate NIR measurements and concentrations.

2.4. NIR spectroscopy

The near-infrared (NIR) region of the electromagnetic spectrum extends from about 800 to 2500 nm wavelength. At shorter wavelengths, the NIR is adjacent to the end of the visible spectrum, which ranges from 380 to 750 nm. At longer wavelengths, the NIR is bordering the beginning of the mid-infrared (MIR, 2500–30,000 nm) region. Near-infrared spectroscopy exploits the fact that specific molecular structures absorb a characteristic wavelength/frequency of the NIR spectrum. The radiation excites molecular vibrations, but only if there is a change in dipole moment during the vibration such as in –CH, –NH, –OH functional groups. If the frequency of the radiation absorbed equals the frequency of vibration of the molecular bond, a so-called 'fundamental vibration' occurs. The fundamental vibrations are typical for the mid-infrared region. The higher frequency of NIR radiation, however, excites so-called 'overtones' with frequencies approximately two (i.e., first overtone), three (i.e., second overtone) or more times the fundamental vibration. In addition, combination bands of fundamental vibrations are typical for the NIR region [4]. The molecular overtone and combination bands in NIR spectra lead to broad and overlapping peaks, which are unsuitable for the identification of compounds. The primary benefit of NIR spectroscopy unfolds when used for quantitative analyses computed from calibration models. Therefore, multivariate (multi-wavelength) calibration with known reference data (e.g., chemical composition) is necessary.

The instrument used in this work is a Brimrose Luminar 5030 NIR spectrometer: The light from a tungsten–halogen lamp is directed into a TeO_2 crystal. The transmitted wavelength of light is determined by the radio frequency applied to the crystal, which acts as acousto-optic tunable filter (AOTF) and allows fast scanning of the entire wavelength range. A single spectrum, covering the wavelength range 1100–2300 at 5 nm intervals, is acquired in only 17 ms. The monochromatic light is passed through fiber optics to illuminate the sample; the transmitted or reflected light is passed through another fiber optics to an InGaAs photo detector. Due to the AOTF technology without moving parts, the spectrometer is very rugged; vibrations or shocks will not change the wavelength calibration. These properties are advantageous in industrial applications.