The potential of near infrared spectroscopy to monitor the ethanol fermentation process at a laboratory scale

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Introduction

The technical processes employed to produce biofuel are in an advanced stage of development and several options can be followed.[1] Bioethanol is at present mainly produced by fermentation of easily fermentable sugar sources such as starch substrates (corn, wheat, and others) in North America and Europe, or other high sugar sources, such as beet molasses in Europe and sugar cane in Brazil.[2] In the near future new biomass resources must be sought to produce ethanol by fermentation (2^{nd} generation). At the laboratory scale**,** alcoholic fermentation from starch substrates can be easily monitored by weight loss, corresponding to the CO_2 released during the fermentation process. Zhoa *et al*. (2009) used this technique to monitor the alcoholic fermentation of wheat cultivars.[3]

This method has the advantage of being fast and inexpensive, without any influence on the fermentation process, due to the absence of actual media collection to perform reference analysis such as high performance liquid chromatography (HPLC). Because some other products are generated and consumed during the fermentation process, the development of simple and reliable tools for monitoring the fermentation process without inducing media disturbances is required. Scarff[4] underlines the potentiality of near infrared (NIR) spectrometry to follow biological processes such as fermentation. The methods of collecting spectra can be classified into three types; off-line, at-line and on-line (this last type can be further divided into *in-situ* and *ex-situ*). As an example, Blanco[5] used the at-line technique to develop an NIRS model to assess ethanol, glucose, glycerine and biomass from fermentation of glucose media by S*accharomyces cerevisiae*.

The aim of this study is to investigate, in a simple and inexpensive way (reference values are obtained simply by measuring the weight losses of the fermentation vessel) the potential of NIRS on line *ex-situ*, for monitoring fermentation process at a laboratory scale on a simple model (starch based substrate). This study is a prerequisite for assessing monitoring of alcoholic fermentation by NIR on various compounds (e.g. ethanol glucose, glycerol) based on HPLC as reference method.

Figure 1. Erlenmeyer flask equipped with an aluminium plate used as reflector.

Materials and methods

Hydrolysates (350 g/sample) were obtained from whole-meal wheat (five samples) and wheat starch (five samples). 70 g (dry matter) were treated with the following enzymes; Viscosyme Wheat, Termamyl SC DS and Spirizyme fuel, according to the manufacturer specifications (Novozymes, Denmark).[6–8]

Fermentations of the hydrolysate, inoculated with a *Saccharomyces cerevisiae* strain, were conducted in 1 litre Erlenmeyer flasks. A preliminary study, performed on the flasks at the end of fermentation, showed that it was not possible to collect useful spectra directly through the flask, due to the low media reflectance. From that time, the flasks were equipped with an aluminium plate (3.8 cm × 10.9 cm) which was used as a reflector, designed to cover the bottom of the flasks (Figure 1).

The fermentation process was conducted in an orbital incubator at 32°C, and 80 rpm for 48 hours. At different times of incubation (every two hours in the first 24 hours and then at 30, 44 and 48 hours) the flasks were removed from the incubator weighed (at 0.01 g level) and NIR spectra were recorded (three measurements) by placing the flask directly on the sample window of a FOSS XDS scanning monochromator (Figure 2).

The spectra were acquired in transflectance mode in the wavelength region of 400 to 2500 nm. Starch and whole-meal samples were fermented in triplicate, with at least one flask without an aluminium plate, used as control (to assess the effect on the fermentation process of the aluminium plate, and the removing of the flask from the incubator to collect spectra). Weight losses recorded were converted into % (w/w) of ethanol by the following equation: ethanol (% w/w) = (Weight loss/Weight of the medium)*1.045*100. These values were used as reference data for the development of NIR equations.

Figure 2. Erlenmeyer flask spectral acquisition directly on the sample window.

The spectra were acquired by the FOSS Winscan software, data treatment and calibration development were achieved using the FOSS WINISI III software.

Results

Effect of methodology on the fermentation process

The kinetics curves obtained by graphing the weight losses versus time of a sample are presented in Figure 3. This figure shows, for one sample, that the method using aluminium plates for the spectra acquisition did not interfere with the course of the fermentation.

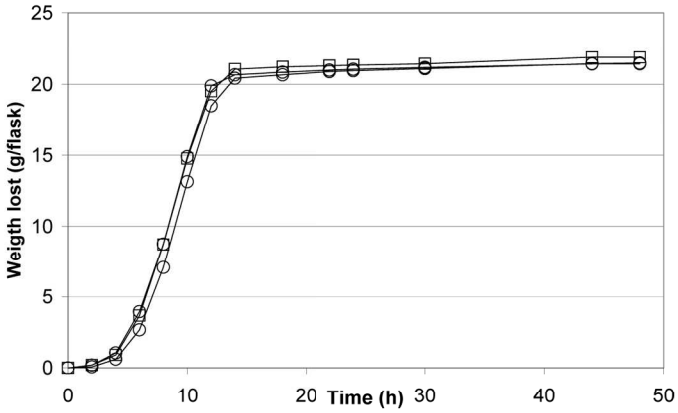

Figure 3. Kinetics of the weight loss. Weight loss kinetics with (dotted $n = 2$) and without (squared $n = 1$) aluminium plate.

Table 1. Characteristics of the fermentation samples regarding the weight losses (WL in g/100 g media) or the ethanol production (EtOH in g/100 g media).

	Calibration set			Validation set		
Constituent	*n*	*SEC*	R^2_C	*n*	*SEP*	*SD/SEP*
WL	215	0.13	0.997	56	0.16	15.6
EtOH	215	0.24	0.995	56	0.28	10.0

Model development

The alcoholic fermentation was monitored using the weight loss and ethanol production. In total 10 samples (three replicates) divided into two batches of fermentation were processed. The weight loss values ranged from 0 to 7.55 g/100 g of media. The data were separated into a calibration set ($n=215$) and a true validation set ($n=56$, including all of the flasks and all of the replicates of the initial wheat whole-meal. A first step-wise calibration trial showed typical wavelengths linked to ethanol at (1690 nm, 2200 nm, 2260–2270 nm). The equations were developed by PLS applying a SNVD treatment and a slight smoothing (0, 0, 5, 1) without any derivatives to the spectral data (1600–1850, 2 nm and 2200–2492, 2 nm). Based on the *SD/SEP* ratio, the best calibration results were obtained while predicting the weight loss (*SEC*=0.13, *SEP*=0.16, *SD/SEP*=15.6). Calibrations were also derived from the calculated ethanol content (*SEC*=0.24, *SEP*=0.28, *SD/SEP*=10.0). A summary of all the results is shown in Table 1.

Conclusion

This study shows the potentiality of an on-line *ex-situ* NIR instrument to monitor laboratory scale fermentation in small glass vessels, by predicting ethanol concentration derived on the basis of weight losses. Further development is required to assess the production of other fermentation substances such as glycerol, organic acids (e.g. lactic and acetic) and biomass. The fermentations can also be assessed by sugar consumption (e.g. glucose, maltose). These components must be determined by chromatographic methods such HPLC for relevant reference values. If a reliable NIR model based on these parameters can be developed, it will be a powerful tool for monitoring the fermentation process. Such a tool could be used at a laboratory scale in small size vessels for screening and selecting the best microorganisms and/or substrates for second generation bioethanol production.

References

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