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MONITORING THE PHENOLICS COMPOUNDS OF THE 2G ETHANOL PROCESS

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ABSTRACT

One of the main challenges in the production of second generation (2G) ethanol is related to characterization and monitoring of hydrolyzates from lignocellulosic biomass, which often contain high quantities of phenolic compounds that inhibits both enzyme and microbial activity. Rapid, efficient, and low-cost technologies for monitoring the phenolic compounds during 2G ethanol production are essential for better control of the pretreatment, hydrolysis and fermentation process. This paper reports on the viability of monitoring phenolic compounds by a spectrometric technique (region of Ultraviolet-Visible – UV-Vis) associated with Partial Least Squares (PLS) regression. For that, synthetic samples were considered in PLS-UV-Vis models (Vanillin, gallic and p-coumaric acids). The best predicting concentrations provided satisfactory accuracy by presenting PLS-UV-Vis models with root mean square error of prediction in cross validation (leave-one-out, LOO) around 8% (RMSECV). Our results indicate that the PLS-UV-Vis models were applicable for monitoring gallic acid in the samples.

1. INTRODUCTION

High amounts of weak acids, furan derivatives and phenolic compounds are released from lignocellulosic biomass during the pretreatment step of the production of 2G ethanol (Jönsson and Martín, 2016). Among the compounds in the hydrolyzate of biomass, the phenolic compounds inhibits and/or deactivates the biological catalysts of the enzymatic hydrolysis reaction (Jönsson and Martín, 2016). Monitoring these phenolic compounds could help to overcome these bioprocesses limitations.

Rapid, efficient, and low-cost technologies for monitoring the inhibitory compounds of the hydrolyzate are essential for the large-scale production of 2G ethanol. Partial Least Squares (PLS) associated with samples spectrums enable a fast and no destructive quantification of multiconstituents mixtures with few, or none, sample preparation. This technique has been applied for the quantification of ethanol and glucose in fermentation broths (Pinto et al., 2016) as well as for quantification of phenolic acids in fruit juices (Khani et al., 2016). Here, a methodology for monitoring some of the phenolic compounds found in the hydrolyzates of sugarcane bagasse was investigated by using a set of synthetic samples and PLS-UV-Vis.

2. MATERIALS AND METHODS

2.1. Synthetic Samples

Different concentrations of synthetic samples were prepared from the solubilization in water : ethanol of three phenolic compounds (Vanillin, Gallic acid and p-coumaric acids). The solubilization of the analytes was standardized using solutions of water: ethanol in proportions that made possible the complete solubilization of these compounds in the concentration of 5 g/L (vanillin: 0.17 kg of ethanol per 1 kg of solution; gallic acid: 0.17 kg of ethanol per 1kg of solution, p-coumaric acid: 0.36 kg of ethanol per 1kg of solution). The analytes in these samples were purchased from Sigma Aldrich and Impex Ltda (Brazil) with HPLC standard purity.

2.2. UV-Vis Spectroscopy

Off-line UV-Vis spectra were collected using a UV-Vis from Shimadzu (UV-1600, software UVProbe 2.31- quartz bucket) spectrometer (200 to 600 nm by the resolution of 0.50 nm at $\bar{T}$~28°C).

2.3. Multivariate Analyses

The samples spectra were pre-processing by first and second derivatives (Deriv.) (Savitzky and Golay, 1964) plus mean centering of the absorbance data (MC). A second order polynomial approximation using 11 data points were considered for the derivatives. Identification and removal of outliers samples was performed by analyzing the Leverage and Student Residues (Ferreira et al., 1999). PLS regression was the multivariate linear regression. The number of latent variables (LV) were defined by the conventional cross-validation LOO. The performance of PLS models were evaluated by the root mean square error of prediction in cross validation (RMSECV) and root mean square error of prediction in test set (RMSEP) (equation 1) (Brereton, 2007).

$$\boldsymbol{RMSECV^{i}} = \sqrt{\frac{1}{ac_{cal.}}\sum_{k=1}^{ac_{cal.}}\left(\boldsymbol{c_k^{(i)}} - \boldsymbol{\hat{c}_k^{(i)}}\right)^2} \quad (1)$$

For the i-th analyte, $ac_{cal.}$ is the amount of calibration data set, $\boldsymbol{c_k^{(i)}}$ is the reference $\boldsymbol{\hat{c}_k^{(i)}}$ is the predicted value of concentrations in k-th sample in cross validation. The RMSEP calculation is analogous, except for considering the test data. All procedures for the refinement and pre-treatment of the spectral, as well as the necessary statistical analysis, were implemented in Matlab® (PLS tool box).

3. RESULTS AND DISCUSSION

The PLS-UV-Vis models were based on both calibration and test sets, after removing the outliers. Table 1 shows the data samples considered in the PLS regression models.

Table 1 - Validity range, mean, standard deviation (SD), the number of samples (NS) used in the calibration and validation sets of PLS-UV-Vis models with LHW data.

Chemical Compounds	Concentration of calibration data set(g/L)				Concentration of test data set(g/L)			
	NS= 103				NS=9			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
Vanillin	0	0.050	0.0023	0.0084	0	0.012	0.0015	0.0040
Gallic acid	0	0.018	0.0018	0.0042	0	0.010	0.0017	0.0033
P-coumaric acid	0	0.003	0.0002	0.0006	0	0.000	0.0000	0.0000

A complete evaluation of the performance of the models, cited in the methodology (see section 2.3), was carried out by applying different combinations of pre-processing. The best result of PLS-UV-Vis models and respective spectral pre-treatments is presented in Table 2.

The quantification of gallic acid presented RMECV of approximately 8%. Since the precision was 0.08 g/L (Range Error Ratio ~ 12.0), this model allows quantification of this acid in the synthetic medium. It was observed a variation in the viability of the PLS models, suggesting that interferences in the solutions (mixture of the analytes, for example) can affect the quantification of certain chemical compounds, as was observed in the models obtained for the vanillin and p-coumaric acid.

Table 2 – Pre-processing and performance parameters of PLS-UV-Vis monitoring for synthetic samples.

Pre-processing	Chemical Compounds	LV	RMSE		RMSECV. C_{MAX}^{-1} (%)
			RMSECV (g/L)	RMSEP (g/L)	
1ª Deriv.; MC	Vanillin	5	0.0076	0.0055	15.28
	Gallic acid	3	0.0015	0.0005	8.42
	P-coumaric acid	5	0.0004	0.0002	11.72

These results suggest that the use of the PLS-UV-Vis methodology for monitoring real hydrolyzate samples should be possible for some analytes.

4. CONCLUSION

The results showed the feasibility of using UV-Vis spectroscopy in combination with PLS regression for monitoring phenolic compounds, such as gallic acid, present in the hydrolyzates originated from sugarcane biomass during 2G ethanol production.

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