USE OF NEAR-INFRARED SPECTROSCOPY FOR ONLINE MONITORING OF ALCOHOLIC FERMENTATION: INFLUENCE OF DIFFERENT YEAST STRAINS

Homero A. Neto[1*], João P. M. Souza[1,2], Charles D. F. Jesus[1], Teresa C. Zangirolami[1,3], Christian O. Martins[1] & Marcelo P. A. Ribeiro [1,3]

[1] *Chemical Engineering Graduate Program (PPGEQ)/ Federal University of São Carlos, São Carlos - SP, Brazil.*
[2] *Bioprocess Engineering/Institute of Natural Resources (IRN) Federal university of Itajubá, Itajubá - MG, Brazil.*
[3] *Chemical Engineering/Department of Chemical Engineering (DEQ)/Federal University of São Carlos, São Carlos - SP, Brazil.*
** homeroneto@estudante.ufscar.br*

ABSTRACT

Growing energy demand and greenhouse gas concerns drive the search for renewable, sustainable, and economically viable energy sources. Ethanol, a sustainable alternative, diversifies the global energy matrix. In Brazil, despite a mature ethanol industry, real-time measurement of key fermentation variables like substrate concentration is challenging, often relying on at-line or offline techniques. Near-infrared (NIR) spectroscopy, widely used in fermentation monitoring, lacks studies on its calibration impact from different yeast strains. This study examines the effects of yeast strains on NIR spectra in alcoholic fermentation. Using sugarcane molasses and two yeast strains, fermentations were conducted under controlled conditions and validated by high-performance liquid chromatography (HPLC). Pre-treatments such as EMSC and autoscaling, along with RMSECV for model optimization, were employed. Results showed no significant impact of yeast strains on NIR spectra, demonstrating effective online monitoring of sugarcane molasses fermentation, with an excellent predictive calibration model evidenced by low RMSECV value (5.2 g/L) and low RMSEP value (6.6 g/L) in external datasets.

Keywords: Alcoholic fermentation. Monitoring. NIR spectroscopy. Yeast strains. TRS.

1 INTRODUCTION

Growing energy demand and concerns about greenhouse gases (GHG) emissions fuel the search for renewable, sustainable and economically viable alternative energy sources[1]. Ethanol is a biofuel with great potential to replace fossil fuels and significantly reduce GHG emissions. Brazil is the world's second largest producer of fuel ethanol (28% of global production), second only to the United States, producing 15,620 million of gallons in 2023[2].

In Brazil, the main raw material used to produce ethanol is sugarcane, and ethanol obtained from sugarcane reduces the emission of GHG by around 89% compared to gasoline[3]. In the sugar and alcohol industries, ethanol production can be divided into the following stages: extraction, treatment, fermentation and cogeneration of steam and energy. The production of ethanol from sugarcane is deemed efficient because of the high yields and productivity achieved through a well-established process involving the yeast *Saccharomyces cerevisiae*. Furthermore, most distilleries use the fed-batch process for yeast recycling fermentation, a process known as Melle-Boinot. The great advantage of this process is that it allows fermentation to operate with high concentrations of yeast cells, avoiding the consumption of sugars for yeast growth and budding at the beginning of each cycle, which reduces the time and costs associated with preparing the inoculum and increases the overall efficiency of the fermentation process[4].

Despite Brazil's mature ethanol industry, there remains considerable potential for ongoing advancements. One of the problems associated with the process is the difficulty in measuring in real time important fermentation variables, such as substrate concentration, requiring at-line and/or offline techniques for their determination, such as High-Performance Liquid Chromatography (HPLC). However, the use of HPLC makes real-time monitoring unfeasible, preventing the bioprocess from being optimized and controlled industrially due to the time spent analyzing samples.

Thus, through the fourth industrial revolution, or IR 4.0, the integration of various technologies such as the Internet of Things (IoT), artificial intelligence (AI), robotics, and big data analytics can optimize production processes and improve efficiency[5]. Considering these aspects, the application of techniques that allow monitoring of the process based on direct information acquired quickly, accurately and reliably becomes critical. The NIR spectroscopy technique, near infrared spectroscopy, is a well-established technology that has been widely used to monitor various manufacturing processes fermentation on laboratory and pilot scales[6]. It is based on the energy absorption property of a molecule in a given region of the electromagnetic spectrum, notably at wavelengths from 780 to 2500 nm[7]. It´s main advantage over conventional chemical analyzes is the ability to quantify multiconstituents with minimal, or no, preparation sample, quickly and non-destructively.

In the sugar, alcohol and wine industry, different types of yeast strains are used depending on the conditions and process objectives. The use of different yeasts strains usually results in wines with different sensory properties, despite being obtained from the same grape variety. Some non-*Saccharomyces* species have recently gained attention due to their ability to produce various metabolites of oenological interest[8]. In the Brazilian sugar and alcohol industry, some yeast strains are selected because they are highly tolerant to fermentation stress, persistent, do not flocculate, do not foam and are dominant in relation to contaminants.

The use of NIR spectra has already been applied to the quantification of compounds present in the alcoholic fermentation medium[9,10]. However, no studies were found in the literature that addressed if different yeast strains can affect the NIR spectra calibration of alcoholic fermentation. In this context, the present work aimed to investigate the influence of yeast strains in alcoholic fermentation with the purpose of developing a calibration for substrate concentration and monitoring it online.

2 MATERIAL & METHODS

Four batch fermentations were carried out with cultivation medium formulated from sugarcane molasses provided by Fermentec (Piracicaba, Brazil). Fermentations 1, 2 and 3 were carried out using the commercial baker's yeast (Biox, Paraguay). Fermentation 4 used Fermel® *Saccharomyces cerevisiae* yeast donated by Fermentec (Piracicaba, Brazil). The reactor used is a stirred tank type with a capacity of 5 L. All fermentations were carried out with temperature control maintained at 32°C, using a thermostated bath.

Spectrum measurements were made with the NIR-Online X-One DN50 equipment (Buchi Brasil LTDA). The wavelength range used was 900 to 1700 nm spaced 5 nm apart. One variable was chosen to be predicted: concentration of TRS (Total Reducing Sugars). The concentration of TRS was determined by High Performance Liquid Chromatography (HPLC). During fermentations, samples of the culture broth were taken to measure TRS concentrations every 30 minutes or 1 hour. NIR spectra was measured every 10 minutes. The NIR spectrophotometer was coupled to the recirculation system in the bioreactor (Figure 1), allowing spectra to be collected in real time during the course of fermentation.

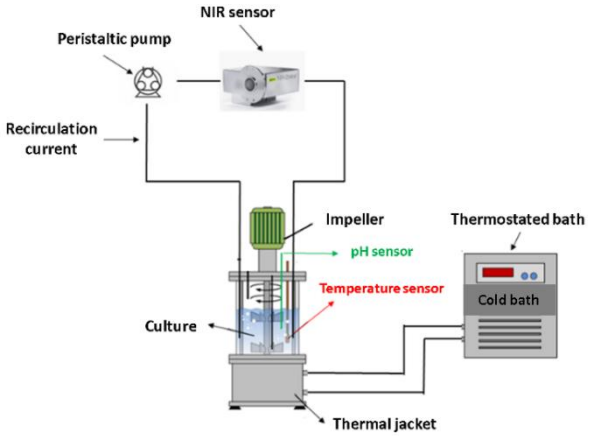

Figure 1 Schematic diagram of the experimental system used to carry out the fermentation tests.

After peaks acquisition, data refinement procedures, statistical analyzes and model adjustment were implemented in the Scilab® software (Scilab 6.1.1). The spectra obtained were pre-processed using EMSC (Extended Multiplicative Scatter Correction) and autoscaling techniques in order to minimize baseline shifts, multiplicative effects, and random variations, highlighting relevant information in the spectrum. The spectroscopic data were correlated with the TRS concentration data from the samples using PLS (Partial Least Squares) regression.

To optimize the model complexity, the k-fold cross-validation method with 5 groups was used and the root mean square error of cross-validation (RMSECV) was calculated for different numbers of latent variables (LV). The number of LV in the model was chosen based on the RMSECV value that did not vary significantly with the increase in the number of LV. Fermentations 1, 2 and 3 were used to calibrate the model (calibration group) and fermentation 4 was used to validate it (external validation group).

3 RESULTS & DISCUSSION

Figure 2 presents the results of cross-validation (blue) with the calibration group and inference from the external validation group (green) for the TRS concentration. The number of LV determined for the optimized model was 4 with a RMSECV value of 5.2 g/L. For the external validation group, the value of the RMSEP (root mean square error of prediction) achieved was 6.6 g/L. These results indicate that the model is robust and can be generalized for external data sets, since the RMSEP value is close to the RMSECV value. It`s worth noticing that the data are randomly distributed around the bisector which indicates a good prediction without large systematic errors.

These findings corroborate with other works in the literature regarding the influence of different yeast strains in NIR spectra of alcoholic fermentation[10], indicating that they don't have strong impact on the calibration of a spectroscopic model. The main influence can be attributed to the usual chemical components found in the alcoholic fermentation, such as ethanol, sugars, and

other chemical components that can be related to the yeast strain used. Some yeast strains are more resistant to contamination, reducing the number of chemical compounds produced by contaminants, such as glycerol, acetic acid, lactic acid. Further studies involving the influence of these contaminants on the NIR spectra of alcoholic fermentation are going to enlighten these questions.

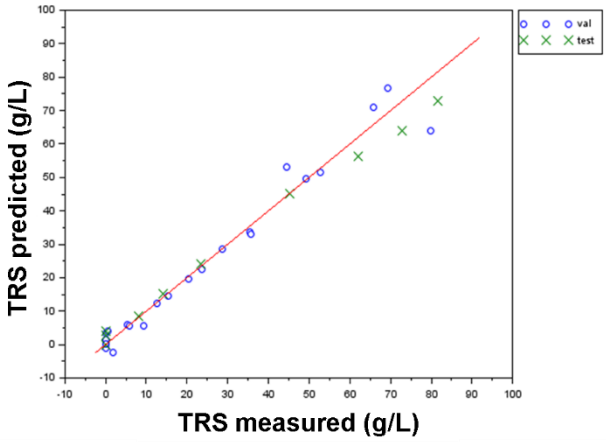

Figure 2 Results of cross-validation (blue) with the calibration group and inference from the external validation group (green) for TRS concentration.

4 CONCLUSION

NIR spectroscopy showed good results for the online monitoring of sugarcane juice fermentation, with an excellent predictive capability of the calibration model, evidenced by the low RMSECV of 5.2 g/L and the RMSEP value of 6.6 g/L in the external dataset. RMSECV values were used to optimize model complexity, achieving a balance between accuracy and simplicity. No significant influence attributed to different yeast strains on the NIR spectra was found, corroborating with other works in the literature regarding the influence of different yeast strains in NIR spectra of alcoholic fermentation. Further studies regarding specific chemical compounds related to yeast strain contamination, such as glycerol, acetic acid, lactic acid, are going to enlighten these questions.

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ACKNOWLEDGEMENTS

The authors would like to thank Fermentec Tecnologias em Açúcar e Álcool LTDA (Piracicaba), FINEP (Financiadora de Estudos e Projetos) and MCTIC (Ministério da Ciência, Tecnologia, Inovações e Comunicações)) with resources from FNDCT (Fundo Nacional de Desenvolvimento Científico e Tecnológico) for financial support (UNIFEI PROEX nº 23088.017409/2022-61 and UFSCar 14.708 – ProEx nº 012827/2022-18). This study was also funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - code 001; FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) - Process 2016/10.636-8; and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Process 140351/2024-8.