

ABSTRACT

Although alcoholic fermentation is well-known and has been used to the benefit of mankind for more than 9000 years; it is still an important topic of research, not only in the food and beverages industry, but also in the fuel sector, due to the feasibility of using it as an ecological, renewable and alternative source of energy in contrast to fossil fuels.

To optimize the production of ethanol a monitoring strategy is needed to keep the process under control. Within this framework lies this PhD thesis with the **main objective of establishing an in-line analytical methodology to follow the alcoholic fermentation process**. Near-Infrared Spectroscopy (NIRS) has been selected as the analytical instrument used to obtain in-line information. To extract useful information from NIR spectra different chemometric approaches have been used. Some of them are widespread algorithms and have been largely contrasted, such as PLS (Partial Least Squares). Others have a high potential, such as MCR-ALS, nevertheless they have never been applied to extract useful information from alcoholic fermentation processes.

In order to accomplish the main purpose, the following steps have been developed:

1. The first step was to establish different methodologies for the determination of the main species involved in the alcoholic fermentation process. Glucose consumption and ethanol formation were enzymatically determined using a UV-VIS (UltraViolet- VISible) detector coupled to a FIA (Flow Injection Analysis) manifold. Biomass accumulation in the culture medium was determined as dry weight method. The formation of glycerine was determined by Gas Chromatography and the progressive acidification of the system was determined by titration. The cited methodologies above provided analytical information that was used as the "foundation" to build the calibration models.
2. Fermentation monitoring was carried out using a NIR instrument furnished with different at-line and in-line modules. In this way, the evolution of the fermentation process was registered and stored as NIR spectra.

3. Partial Least Squares (PLS) was used to correlate the analytical and spectral information, thus, calibration models were obtained for the five key species. These models were externally validated and, consequently, were qualified to monitor the fermentation process.
4. After the construction of PLS models, the feasibility of applying resolution algorithms to unravel the information stored in the spectral data set was checked. MCR-ALS is a powerful tool with low demanding needs in terms of analytical reference information. It turned out that MCR-ALS provided good resolution profiles for glucose, ethanol and biomass.
5. Despite the results obtained with MCR-ALS, a further step was given in order to reduce the inherent ambiguities of the resolution algorithms. In that way, different empirical fermentation models were tested. The model with the higher descriptive ability was selected. The information provided by this model was incorporated into the p-ALS algorithm as an input, in the way of equality constraints. This procedure allowed us to unravel the evolution of the fermentation processes carried out at different conditions of temperature and initial pH. Good results were obtained in terms of determination coefficient between the values provided by PLS and those obtained with MCR-ALS.
6. To finish this work, the effect of temperature in polar liquids was studied. In the above described work, temperature has either been implicitly modelled or its distorted effects have not significantly altered the performance of the models. Yet, it is well known that temperature dramatically affects the performance of calibration models in a negative way when it is not controlled. To solve that problem, an ensemble strategy has been proposed. The method combines the advantages of PARAFAC and MLR to minimize the effect of temperature. PARAFAC is used as a variable reduction method to extract the components that are mainly related to the chemical species but also with the physical effect induced by temperature. MLR is used as a calibration method based on the information provided by PARAFAC and on the reference values. The proposed method was applied to different data sets. The goodness of the figures of merit obtained enables us to state that the effect of temperature was corrected, even in situations of interpolation and extrapolation.