

On-line Monitoring and Control of Fed-batch Fermentations in Winemaking

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Abstract: The fermentation of yeast in fed-batch mode shows great potential in winemaking because it allows the concentration of sugars to be kept low and constant throughout the process which, in turn, reduces cell stress and leads to a significant decrease in the production of unwanted secondary metabolites. The implementation of this technique requires reliable on-line analysis of sugar and a robust control strategy to maintain sugar concentrations at defined levels over the course of the fermentation. In this study, a laboratory-scale setup was used to implement and assess a fully automated fed-batch fermentation of *Saccharomyces cerevisiae* in grape must. Total sugar levels were monitored in-line by FT-MIR ATR spectroscopy and kept constant at 50 g/kg by a modified PI controller regulating the must feed flow rate. Good setpoint tracking and disturbance rejection were achieved in fermentations of up to four days despite occasional yeast sedimentation on the ATR crystal. The controller parameter adaptation strategy needs to be optimized for longer fermentations.

Keywords: Adaptive control · Fed-batch fermentation · Winemaking

Introduction

Winemaking traditionally involves batch fermentation processes where yeast convert the fermentable sugars contained in grape must into alcohol. Major fermentation parameters (temperature, aeration, duration) have been optimized over the course of winemaking history, mostly empirically and based on the sensory evaluation of the final product. However, the quality and sensory attributes of wine are also greatly influenced by the composition of the grape must, which is subject to vintage variations. In recent years, climate change associated acceleration of grape maturation has led to higher grape sugar concentrations. Excessive sugar levels in grape must cause a hyperosmotic stress response in yeast leading to increased formation of undesired fermentation metabolites, such as acetic acid and acetaldehyde, during winemaking.^[1]

Hyperosmotic stress in yeast cells can be minimized by actively maintaining relatively low and constant sugar concentrations in the medium during the fermentation process. This can be achieved by implementing fed-batch fermentations, whereby must is added progressively into the fermentation tank at a rate that corresponds to the metabolic rate of the yeast. Typical sugar

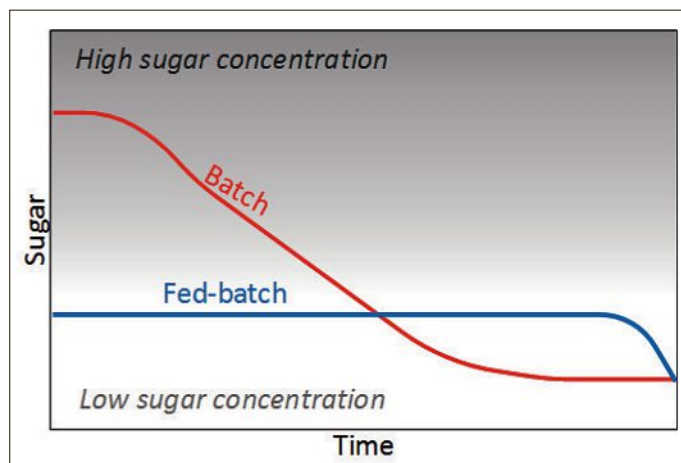


Fig. 1. Typical trajectories of must sugar concentrations during batch and fed-batch fermentations.

concentration profiles in batch and fed-batch modes are shown schematically in Fig. 1.

Frohman *et al.*^[2] noted a significant increase in cell viability and substantially lower acetic acid and acetaldehyde levels in wines produced by the fed-batch technique as opposed to those obtained by using the traditional batch mode. The authors suggested that this improvement was caused by reduced cellular stress and the reutilisation of acetic acid by the yeast. In a follow-up study, Pernet *et al.*^[3] developed a strategy for the in-line monitoring of sugars and ethanol using near-infrared (NIR) spectroscopy. This approach was successfully tested during fed-batch wine fermentations that were automatically controlled to maintain a constant concentration of total sugars.

Besides the need for reliable on-line analysis of sugar levels, the implementation of a fully automated fed-batch system requires a robust control strategy capable of dealing with non-linearly evolving process conditions (in this case, the exponential yeast growth). In this work, closed-loop control of fed-batch wine fermentations was implemented at the laboratory scale using a Fourier-transform mid-infrared (FTIR) spectrometer for the in-line monitoring of the total sugar concentration, and a variation of the PI controller in which the proportional and integral terms are adapted exponentially to account for the specific growth dynamics.^[4] An exemplary experiment is shown and discussed in this paper.

Materials and Methods

The yeast used in this work, *Saccharomyces cerevisiae* strain DV10, is commercially available from Lallemand Inc. (Montreal, Canada). Grape must, originating from the Domaine de Montmollin (Auvèrner, Switzerland), had a total fermentable sugar concentration of 230 g/l and was supplemented with 200 mg/l of diammonium hydrogen phosphate as nitrogen source. The inoculation rate was 0.07 g/l. The fermentations were carried out at 20 °C, with a short start-up batch phase in a small initial volume followed by the fed-batch phase.

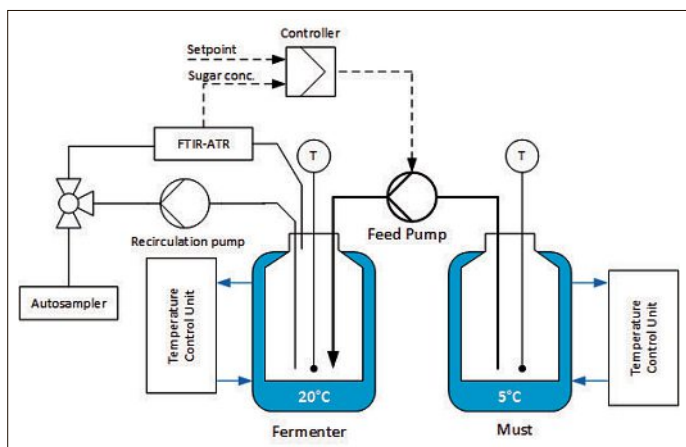


Fig. 2. Process diagram of the experimental setup used for fed-batch Ovine fermentations.

A process diagram of the experimental setup is shown in Fig. 2. Specifically, the must was maintained in a temperature-controlled tank at 5 °C and fed into the fermenter using a peristaltic pump. A second peristaltic pump was used to recirculate the fermentation broth through the FTIR's attenuated total reflectance (ATR) flow cell. Measurements were sent from the FTIR to the controller which, in turn, regulated the speed of the feed pump. An autosampler was used to withdraw broth samples through a three-way valve installed on the recirculation loop for off-line reference analysis. Reference analysis of sugars and ethanol was performed by chromatographic methods (GC and HPLC).

The FTIR spectrometer (PerkinElmer Spectrum One) was calibrated for the analysis of total sugars (glucose and fructose) and ethanol using standards prepared following a 7-level partial factorial design for multivariate calibration.^[5] A partial least squares (PLS) model was built and evaluated using external validation. The standard errors of validation (SEV) were 4.5 g/l for total sugars and 2.8 g/l for ethanol.

The flow rate of the feed pump, $F(t)$, was regulated by the following proportional-integral controller:

$$F(t) = F_0 \cdot \exp\left(\mu \cdot t + K_p \cdot e + K_I \cdot \int_0^t e(t) \cdot dt\right) \quad (1)$$

where F_0 is the initial flow rate, μ is the specific growth rate of the yeast, K_p and K_I are the proportional and integral gains, respectively, and $e(t)$ is the control error. The initial flow rate was calculated based on the specific growth rate and the concentration of yeast in the fermenter at the beginning of the fed-batch phase. The values of the controller gains were tuned empirically.

Results and Discussion

A four-day fermentation was carried out producing approximately 3 litres of wine. Fig. 3 shows the concentration profile of sugars during the fed-batch phase for a constant setpoint of 50 g/kg, as determined in-line by FTIR.

The system showed good setpoint tracking and recovery following three major disturbances. The first disturbance, at fermentation time of 0.9 days, was caused by the replacement of the initial 250 ml fermentation tank with a larger one to accommodate the growing volume.

The following two disturbances, occurring after 1.5 and 2.6 days of fermentation time, were caused by the accumulation of

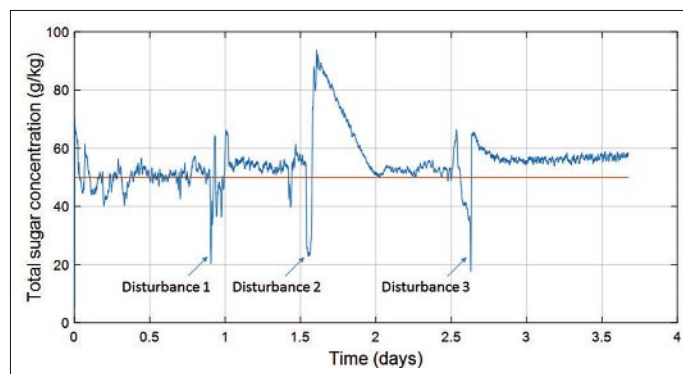


Fig. 3. Profile of total sugar concentration during fed-batch fermentation of grape must.

yeast on the ATR window inside the flow cell. The yeast buildup provoked the formation of a small stagnant volume inside which the concentration of sugars dropped. The controller reacted to the false signal of declining sugar levels by increasing the feed flow rate of grape must which resulted in the accumulation of sugars beyond the setpoint value. After detecting the problem, the recirculation loop was momentarily disconnected to allow the flow cell to be cleaned. As can be seen in Fig. 3, the excess sugar in the fermenter was subsequently consumed by the culture and control was re-established.

During the last day of the experiment, a static error of approximately 10% was noted. This was most likely due to the controller gains becoming ineffective with respect to the high biomass concentration towards the end of the fermentation.

Conclusions and Perspectives

The implementation of a fully automated fed-batch process of wine fermentation was achieved at the laboratory scale over a period of four days. In-line monitoring of total sugar levels was implemented by mid-infrared spectroscopy. The control strategy proved successful but the results showed that care must be taken to avoid yeast buildup on the ATR crystal during fermentations. Future work will focus on resolving this issue, improving the robustness of the controller and testing the approach over extended fermentation periods and on a larger scale.

The parameters of the controller should be tuned following a more systematic approach and adapted over the course of the fermentation in order to improve the controller's long-term effectiveness and robustness. Follow-up experiments will consider assessing cell viability, acetic acid and acetaldehyde concentrations, as well as the effect of the proposed methodology on the quality of the produced wine. Finally, for a successful practical implementation of the fed-batch control approach, a low-cost alternative for the measurement of glucose in the must should be developed. Selective captor technology found in clinical instrumentation may be a promising approach.

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- [1] R. Mira de Orduña, *Food Res. Int.* **2010**, *43*, 1844.
- [2] C. Frohman, D. Widmer, R. Mira de Orduña Heidinger, *Revue Suisse Viticulture, Arboriculture, Horticulture* **2014**, *46*, 182.
- [3] A. Pernet, R. Mira de Orduña Heidinger, D. Widmer, A. Riedo, M. Mertenat, M. Cléroux, J.-P. Bourgeois, O. Vorlet, C. Frohmann, *Revue Suisse Viticulture, Arboriculture, Horticulture* **2015**, *47*, 192.
- [4] M. Dabros, M. M. Schuler, I. W. Marison, *Bioproc. Biosyst. Engin.* **2010**, *33*, 1109.
- [5] J. A. Muñoz, R. G. Brereton, *Chemometrics and Intelligent Laboratory Systems* **1998**, *43*, 89.