Dynamical modeling of alcoholic fermentation and its link with nitrogen consumption


Abstract: This paper proposes a dynamical mass balance model describing the main physiological phenomena observed during the batch fermentation in the wine-making process, on the basis of a set of biological reactions in which the nitrogen consumption plays a central role, in line with experimental evidence deduced from extensive experimental studies (9). The experimental database considered for the parameters identification has been generated by a simulator issued from a logistic model especially dedicated to the wine fermentation (10) which is a valuable representation of the process, yet with a complex formulation that appears to be not suitable for control purposes. The paper presents the results of the modeling efforts performed on the basis of this model so as to include in particular the influence of nitrogen on the key bio-mechanisms involved during the fermentation.

Keywords: Wine-making, fermentation, batch reactor, dynamical model, nitrogen, glucose transporters, yeast activity

1. INTRODUCTION

The food industry is well established and many processes in operation nowadays are the subject of intensive work with regard to the ways of devising better operation modes in terms of product quality and safety (how to operate in order to ensure quality and comply with safety constraints) as well as in terms of operation costs and environmental impact. Among the four categories (namely bioconversion, separation, preservation and structuring (3)) of food processes, bioconversion includes probably the largest class of processes. It is central in the production of fermented foods with many well known examples from the dairy or winemaking industry, but is also the purpose of cooking in its more general conception. Understanding the underlying kinetic mechanisms and developing models of use in sensing technologies and control are crucial aspects of these type of processes. Models and simulation tools are required to develop efficient control algorithms to maximize the products quality (1).

The development of paradigms in food process control is one of the issues of the EC CAFE project (www.cafe-project.org) that considers four case studies, and among them the bioconversion process of wine-making mainly described by the alcoholic fermentation step.

The objective of the CAFE project about the wine-making fermentation is to design control tools aimed at optimising the fermentation so as to obtain a well defined aromatic profile. Indeed, during the alcoholic fermentation hexoses are converted to ethanol and carbon dioxide, but many other compounds are removed from the must and a large set of by-products are formed that affect the organoleptic properties of the wine. These by-products (higher alcohols, esters, sulphur compounds) represent less than 4 % of the yeast production whereas the ethanol and the glycerol represent respectively more than 90 % and around 5 % of the production.

Different approaches can be considered to provide reliable information about the ethanolic fermentation process dynamics. A first a priori classical modelling approach is to consider mass balances of the key components combined with an appropriate knowledge about the process operation and dynamics. Another a priori attractive approach may be to consider microbiological knowledge about the production of the important organoleptic compounds of the wine in order to develop models that might link the metabololc pathways knowledge to the process operation. If the second approach remains a challenging task (e.g.(5)), the first approach has been the subject of extensive studies over the past decades.

The first comprehensive kinetic models (2)(4)(11)(12)(13) were describing the influence of sugar and ethanol levels, and of temperature on sugar utilization, capturing the general macroscopic trends found in practice. Models have been developed to predict the transition from normal
to sluggish or stuck fermentation, with kinetics based on nitrogen as a growth-limiting nutrient, in isothermal conditions (7), and later by including the temperature dependency of some parameters (6). Unfortunately, the relevance of these models have not been validated in real wine-making conditions.

Several more empirical or non-parametric models have also been published and the model of Malherbe et al. (10), largely based on the first principle modeling approach, considers the main yeast physiological mechanisms. This model consists of ordinary differential equations including numerous parameters that need to be identified and important interactions between explanatory variables. The model accurately predicts the fermentation kinetics of more than 80% of a large number of experiments performed with 20 wine yeast strains, 69 musts and different fermentation conditions.

This kinetic model is based on the main physiological mechanisms limiting the yeast activity. This latter is implicitly described by four subsystems:

1. glucose transport, inhibited by ethanol $E(t)$ and the limiting feature of fermentation;
2. glycolysis, i.e. glucose degradation into ethanol and $CO_2$, which is not limiting;
3. nitrogen transport, also strongly inhibited by ethanol;
4. synthesis of glucose transporters from a fraction of the absorbed nitrogen.

The model predicts the rate at which glucose is consumed and the amount of ethanol or $CO_2$ produced. It includes the effects of the main involved factors: temperature $T(t)$, which can vary within a predefined range (18 to 30°C) and assimilable nitrogen $N(t)$, which has a major impact on the yeast activity and varies a lot according to the musts. This latter consideration is here primordial as nitrogen is the source of the flavour-compounds precursors and the limiting nutrient of the alcoholic fermentation.

However this model does not include consistent mass balances and is not easy to manipulate as the equations formulation is complex and the large number of parameters does not allow a straightforward identification (if a modification due to operational conditions is necessary). Nevertheless the resulting simulator can be used to produce an easy-to-use experimental database, given the significant results provided by this model and the involved knowledge of the process it required.

The next section gives an overview of the modeling effort: the development and the validation of the two models are respectively presented in Section 2.1 and 2.2. Some conclusions are drawn in Section 3.

2. MASS BALANCE MODELING

The objective is to progressively derive a mass balance model which is based on a set of biological reactions and is able to describe the behaviour of the batch fermentation process.

1 Between one and two weeks are typically necessary to achieve a complete fermentation, but the process can take significantly longer to complete (sluggish fermentation) or can leave an important residual sugar level (stuck fermentation).

### 2.1 First Model

A first reaction scheme is considered where biomass $X$ grows on nitrogen $N$ (the limiting nutrient in the fermentation process), and sugar $S$ is enzymatically degraded into ethanol $E$ and $CO_2$, and inhibited by ethanol:

\[
\begin{align*}
    k_1 N & \rightarrow X \\
    k_2 S & \rightarrow E + CO_2
\end{align*}
\]

where the coefficients $k_1$ and $k_2$ represent the yield coefficients respectively associated to nitrogen and sugar consumptions.

The work of Malherbe et al. (10) suggests that the concentrations of glucose $S(t)$ and ethanol $E(t)$, and their time variations $S$ and $E$ can be deduced from the released amount of carbon dioxide $CO_2(t)$ assuming Gay-Lussac-like relations (8):

\[
\begin{align*}
    S(t) &= S(0) - 2.17 CO_2(t), \text{ with } CO_2(0) = 0 \quad (3) \\
    E(t) &= 0.464 (S(0) - S(t)), \text{ with } E(0) = 0 \quad (4)
\end{align*}
\]

leading to

\[
E(t) = CO_2(t) \iff E(t) = CO_2(t) \quad (5)
\]

\[
\dot{S}(t) = -2.17 CO_2(t) \quad (6)
\]

The kinetics are written using the classical formulation of Michaelis-Menten:

\[
\begin{align*}
    \dot{X} &= \mu_{max}(T) \frac{N}{K_N + N} X \\
    \dot{N} &= -k_1 \mu_{max}(T) \frac{N}{K_N + N} X \\
    \dot{E} &= CO_2 = \beta_{max}(T) \frac{S}{K_S + S} \frac{K_E(T)}{K_E(T) + E} X \\
    \dot{S} &= -k_2 \beta_{max}(T) \frac{S}{K_S + S} \frac{K_E(T)}{K_E(T) + E} X
\end{align*}
\]

with

\[
\begin{align*}
    X_0 &= X(0), & E_0 &= CO_2(0) = 0, \\
    N_0 &= N(0), & S_0 &= S(0),
\end{align*}
\]

where $\mu_{max}(T)$ and $\beta_{max}(T)$ are the maximum specific reaction rates; $K_N$ and $K_S$ are the Michaelis (or half-saturation) constants, and $K_E(T)$ represents the ethanol inhibition.

The parameters have been identified using the Nelder-Mead (simplex) method implemented in the Matlab function fminsearch, and the ODE solver ode15s. The model has a cascade structure which makes easier the identification task: the parameters $\mu_{max}(T)$, $K_N$ and $k_1$ are identified before $\beta_{max}(T)$, $K_S$, $K_E$ and $k_2$.

Results are shown in Figures 1, 2 where curves from the model (7) (*iden) are fitted with data from the model of Malherbe (*data).

Parameters have been identified with data generated from $N_0 = 0.17$ g/l and $T = [18.2:30]°C$. Figures 1 and 2 show
the results for two different temperatures: \( T = 30^\circ C \) for direct validation and \( T = 25^\circ C \) for cross-validation.

Attention has been particularly paid to the \( CO_2 \) production rate \( CO_2 \) during the development of the empirical model because its measurement is easy and precise and furthermore this variable corresponds to the rate of the main reaction of the alcoholic fermentation. It can be observed that the Malherbe-model results are well described by the (simply formulated) mass balance model.

However experiments used to design the Malherbe model have underlined the influence of the initial concentration of nitrogen \( N_0 \) on the batch process. This is illustrated in Figure 3 where a different initial condition on the nitrogen concentration has been considered (\( N_0 = 0.57 \) g/l instead of 0.17 g/l in the two previous figures).

In Figure 4, it can be seen that the maximum value of biomass concentration \( X_{\text{max}} \) does not evolve linearly with \( N_0 \) in practice (light gray curve), as it could be expected and as it is actually illustrated by our first mass balance model (\( \cdots \) curve). Indeed, the model formulation implies a complete consumption of the nitrogen for the biomass growth and consequently a linear relation between \( X_{\text{max}} \) and \( N_0 \), whatever the considered kinetic model (Michaelis-Menten, Haldane, ...)(only their transient behaviour will change). In the model of (10), the initial condition \( N_0 \) is considered as a parameter of the differential equations which cannot be the case in a mass balance design of the process.

\[
\dot{X} = \mu_{\text{max}}(T) \frac{N}{K_N + N + \frac{N^2}{K_1(T)}} X
\]

\[
\dot{N} = -k_1 \mu_{\text{max}}(T) \frac{N}{K_N + N + \frac{N^2}{K_1(T)}} X -k_1 \eta_{\text{max}}(T) N X
\]

\[
\dot{T}_{\text{r}} = \eta_{\text{max}}(T) N X
\]

\[
\dot{E} = \beta_{\text{max}}(T) \frac{S}{K_S + S} \frac{K_E(T)}{K_E(T) + E} X
\]

\[
\dot{S} = -k_2 \beta_{\text{max}}(T) \frac{S}{K_S + S} \frac{K_E(T)}{K_E(T) + E} X
\]

Fig. 5. Simplified diagram of the activity of a yeast (10).

During the fermentation these transport proteins have to sustain a catabolic inactivation by the ethanol. This phenomenon increases with the ethanol concentration and impacts the sugar transport, therefore the fermentation kinetic is slacked (decrease of \( CO_2 \) production rate). Experiments have shown that when the initial nitrogen concentration is low, the yeast mainly focus on the cells production. When \( N_0 \) is larger, the cells production increases but at the same time more nitrogen is used for the transporters synthesis so as to prevent their catabolic inactivation (9).

This effect has also been illustrated in (10) by adding assimilable nitrogen in the fermentor once the biomass is at steady-state: the regrowth is weak but the yeast activity is reboosted (peak of \( CO_2 \) production rate).

Fig. 4. Evolution of the maximum value of biomass \( X_{\text{max}} \) depending on the initial concentration of nitrogen \( N_0 \) with the (10) simulator (black), with the first mass balance model (\( \cdots \) curve) and with the second mass balance model (light gray).

This observation leads us to the introduction of another reaction to characterize the nitrogen consumption into the model.

2.2 Second Model

In wine-making conditions, a part of nitrogen is assimilated so as to synthesize new yeast cells but the remaining part is mostly used in the synthesis and repairing of essential proteins, see Figure 5. Indeed, the yeast cell can assimilate glucose and nitrogen thanks to dedicated proteins called transporters which allow the membrane crossing.
Fig. 1. Evolution of biomass, sugar, ethanol and $CO_2$, and nitrogen with the first model (7) at $T = 30^\circ C$. The vertical lines correspond to the respective instants when $\dot{C}O_2 = \max(\dot{C}O_2)$, see last sub-figure.

Fig. 2. Evolution of biomass, sugar, ethanol and $CO_2$, and nitrogen with the first model (7) at $T = 25^\circ C$. The vertical lines correspond to the respective instants when $\dot{C}O_2 = \max(\dot{C}O_2)$, see last sub-figure.
Fig. 3. Evolution of biomass, sugar, ethanol and CO\textsubscript{2}, and nitrogen with the first model (7) with \(N_0 = 0.57\) g/l. The vertical lines correspond to the respective instants when \(\dot{C}O_2 = \text{max}(\dot{C}O_2)\), see last sub-figure.

with
\[
\begin{align*}
X_0 &= X(0), & E_0 &= CO_{2,0} = 0, \\
N_0 &= N(0), & S_0 &= S(0),
\end{align*}
\]  
(13) where \(\mu_{\text{max}}(T)\), \(\beta_{\text{max}}(T)\) and \(\eta_{\text{max}}(T)\) are the maximum specific reaction rates; \(K_S\) and \(K_I(T)\) are respectively the half-saturation constant and the inhibition constant of the Haldane law; \(K_E\) is the Michaelis (or half-saturation) constant; \(K_E(T)\) represents the ethanol inhibition.

It must be noted that no measurements are given for \(T_r\) in the literature or in the Malherbe model and consequently \(k'_1\) is undetermined.

At this stage of the development, \(T_r\) is considered as an output variable allowing the fitting of \(X\) for different initial concentrations of nitrogen. Indeed, a first parameter-identification run shows that the best curves fitting corresponds to an Haldane kinetics for the biomass growth and to a linear growth law for \(T_r\). Figure 6 stresses that these growth laws are very different and explain the totally different objectives in the use of nitrogen:

- when \(N_0\) has a low value (< 0.17 g/l), \(\mu\) is larger than \(\eta\) and therefore \(X\) grows by consuming quickly almost all the nitrogen;
- when \(N_0\) has a large value, \(\eta\) is larger than \(\mu\) and \(T_r\) consumes quickly nitrogen when compared to \(X\). This impacts the value of \(X_{\text{max}}\) as it is like if \(X\) had a lower value than \(N_0\) to start its growth.

These considerations are also illustrated by the resulting light gray curve in Fig. 4 (\(X_{\text{max,Model2.0}}\)) that follows the evolution of \(X_{\text{max, data}}\), showing that for \(N_0 < 0.17\) g/l \(X_{\text{max}}\) evolves like if there is only one reaction of consumption of nitrogen (biomass growth). For \(N_0 > 0.17\) g/l \(X_{\text{max,Model2.0}}\) evolves like if \(N_0\) was just slightly increasing but as a matter of fact a large part of \(N_0\) has been used to synthesize \(T_r\).

Furthermore, the nonlinear increase of \(X_{\text{max}}\) illustrated in Fig. 4 (black) and extrapolated by a second order...
polymer must be compensated by the increase of the steady-state concentration of transporters $T_r^{\text{max}}$ so as to obtain a complete consumption of nitrogen, i.e., a linear relation between $N_0$ and the total concentration of the products issued from nitrogen. This means that the addition of the curves of $T_r^{\text{max}}$ and $X_r^{\text{max}}$ should result in a linear relation function of $N_0$. Unfortunately, information about the transporters $T_r$ is missing:

- $T_r^{\text{max}}(N_0)$ is needed so as to determine this full consumption curve;
- the transient behaviour determined by the $\eta^{\text{max}}$ law must be somehow validated so that the kinetic term $\eta$ is not meaningless.

This means that new assumptions will have to be developed in the future so as to bypass these difficulties.

In comparison with the model of (10), the mass balance model has also to predict the time evolution of the fermentation when there is an addition of nitrogen at the beginning or on half way of the fermentation. This issue needs further development because two mechanisms work in parallel: the observed peak of yeast activity is originated by the biomass regrowth and/or by the reboosted existing cells.

3. CONCLUSION

The wine-making fermentation involves complex biochemical mechanisms and an efficient mass balance modeling of the key components requires a relevant experimental database. This experiment background has been synthesized in the logistic model of (10) and the resulting simulator provides a representative behaviour of the process. Nevertheless this model is not suitable for the control purposes invoked in the CAFE project as it is not easy to manipulate and adapt (numerous parameters and initial conditions used as parameters). A mass balance model is therefore developed by using the easy-to-use database provided by the Malherbe et al. simulator.

A first model has been developed that suitably describes the fermentation for different temperatures but not for different initial nitrogen concentrations. Indeed the consumption of nitrogen is split into two terms, biomass growth and transport proteins synthesis, this latter increasing with the initial concentration of nitrogen $N_0$. A second model taking account of both nitrogen uses has been designed and successfully validated with a preliminary identification run. A next step can be completed by integrating the effect of a nitrogen addition during the fermentation and this will be the subject of further developments.

ACKNOWLEDGEMENTS

This paper includes results of the CAFE project that is supported by the Food, Agriculture and Fisheries, and Biotechnology program of the European Community (Contract number KBBE-212754). It also presents research results of the Belgian Programme on Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister’s Office, Science, Technology and Culture. The scientific responsibility rests with its authors.

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