

Modeling of the boost effect originated by nitrogen addition during wine-making fermentation

Robert David, Denis Dochain*, Jean-Roch Mouret, Alain Vande Wouwer and Jean-Marie Sablayrolles

Abstract—The main physiological phenomena observed during the grape-must fermentation have been modelled based on a set of biological reactions in which nitrogen use is a major phenomenon. Moreover, a common practice in wine-making is the addition of nitrogen during the batch fermentation so as to boost and shorten the process duration. A tractable representation of this boost effect has therefore been developed and validated with experimental data.

I. INTRODUCTION

Grape-must fermentation by yeast allows the transformation of sugar into ethanol and determines most of the aromatic profile of the wine. The results issued from the EC CAFÉ project¹ (www.cafe-project.org) could hopefully optimize these organoleptic properties thanks to

- a better understanding of the fermentation kinetics through the development of mathematical models;
- the design of efficient control algorithms.

The first step in this bioconversion study is the development of a reliable simulator describing the main process kinetics.

As mentioned in a previous paper [1], the first comprehensive kinetic models ([2], [3], [4], [5], [6]) 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 also been developed with kinetics based on nitrogen as a growth-limiting nutrient, in isothermal conditions [7], and later by including the temperature dependency of some parameters [8]. Unfortunately, the relevance of these models has never been validated in real wine-making conditions.

Several more empirical or non-parametric models have also been published and among them the most validated in the wine context is the model of Malherbe *et al.* [9] that considers the main yeast physiological mechanisms by predicting the rate at which glucose is consumed and the amount of ethanol or CO₂ 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. This model unfortunately does not include consistent mass balances. With that respect its extension to new compounds (aromatic markers, oxygen) is difficult because 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).

This article presents a comprehensive kinetic model that has been validated on numerous experimental data and that outperforms the before-mentioned models.

The next section presents the development of the model for standard fermentations whereas Section III describes the model extension so as to include nitrogen addition during the fermentation. Some conclusions are drawn in Section IV.

II. FERMENTATIONS WITHOUT NITROGEN ADDITION

The reaction scheme commonly used in fermentation considers the growth of biomass on nitrogen and sugar, and the synthesis of ethanol from sugar [10]. The literature review and experimental observations have led us to the conclusion that the first step in the fermentation is the catalyst synthesis, namely the growth of yeast with the nitrogen amount as limiting substrate. The second one is the catalysis, namely the degradation of the non-limiting sugar substrate into ethanol and carbon dioxide. Nevertheless it has been observed [1] that nitrogen is not only consumed for the growth of biomass but that another important reaction is involved.

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. Indeed, the yeast cell can assimilate glucose and nitrogen thanks to dedicated proteins called transporters which allow the membrane crossing. 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, hence the fermentation kinetic is slowed (decrease of CO₂ production rate). Experiments have shown that when the initial nitrogen concentration N_0 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 [11]. This effect has also been experimentally illustrated by adding assimilable nitrogen in the fermentor once the biomass is at

R. David and D. Dochain are with ICTEAM Institute, UCL, 4 Avenue Georges Lemaître, Louvain-la-Neuve, Belgium {robert.david, denis.dochain}@uclouvain.be

J.-R. Mouret and J.-M. Sablayrolles are with UMR 1083 SPO, INRA SUPAGRO, 2 Place Viala, 34060 Montpellier cedex, France {mouretj, sablayro}@supagro.inra.fr

A. Vande Wouwer is with Automatic Control Laboratory, UMONS, 31 Boulevard Dolez, Mons, Belgium alain.vandewouwer@umons.ac.be

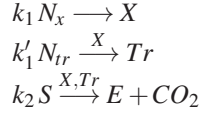
* Honorary Research Director FNRS, Belgium

¹This project aims to maximize the product quality in four typical food processes involving respectively structuring, preservation, separation and bioconversion (wine-making process).

steady-state: the regrowth is weak but the yeast activity is reboosted (peak of CO_2 production rate $\dot{C}O_2(t)$) [12]. This aspect is more largely developed in the Section III.

A. Model equations

The above-mentioned considerations can be summarized in the reaction scheme



where the biomass X (yeast) grows on nitrogen dedicated to the biomass growth N_x . Tr represents the concentration of glucose transporters in excess, as it is explained in the assumptions (Section II-B). N_{Tr} is the nitrogen dedicated to the excess-transporters synthesis. Sugar S is enzymatically degraded into ethanol E and carbon dioxide CO_2 .

It has also been demonstrated in [13] that

$$E(t) = CO_2(t) \iff \dot{E}(t) = \dot{C}O_2(t) \quad (1)$$

$$\dot{S}(t) = -2.17 \dot{C}O_2(t) \quad (2)$$

The cumulated results of [1] [13] and the validation provided by the experiments presented in figure 3 (see Section II-C) have finally led to this ultimate set of mass balances:

$$\left\{ \begin{array}{l} \dot{X} = \mu_{max}(T) \frac{N_x}{K_x X + N_x} X \quad \text{and} \quad \dot{N}_x = -k_1 \dot{X} \\ \dot{Tr} = \eta_{max}(T) \frac{N_{Tr}}{K_{Tr} + N_{Tr}} X \quad \text{and} \quad \dot{N}_{Tr} = -k'_1 \dot{Tr} \\ \dot{E} = \dot{C}O_2 = \left(\beta_{max,1}(T) \frac{N_x}{K_x X + N_x} + \beta_{max,2}(T) \frac{K_E(T)}{K_E(T) + E} \right) \frac{S}{K_S(T) + S} X (1 + \phi(T) Tr) \\ \dot{S} = -k_2 \dot{E} \end{array} \right. \quad (3)$$

with

$$\begin{aligned} X_0 &= X(t=0), & N_{x,0} &= N_x(t=0), \\ Tr_0 &= 0, & N_{Tr,0} &= N_{Tr}(t=0), \\ E_0 &= CO_{2,0} = 0, & S_0 &= S(t=0) \end{aligned}$$

$\mu_{max}(T)$, $\eta_{max}(T)$ and $\beta_{max}(T)$ are the maximum specific reaction rates. K_x is the Contois constant, and K_{Tr} , K_S are the Michaelis-Menten constants. $K_E(T)$ represents the ethanol inhibition. k_1 , k'_1 and k_2 are stoichiometric coefficients. k_2 is equal to 2.17, see (1)-(2). $\phi(T)$ is an efficiency parameter related to Tr and associated to the fermentation activity (ethanol or CO_2 production rate). The (total) initial nitrogen concentration is defined as $N_0 = N_{x,0} + N_{Tr,0}$.

B. Model assumptions

Assumptions had to be formulated about the dynamical behaviour of the variable Tr . No information can be found in the literature and it was necessary to define the substrate distribution into N_x and N_{Tr} , the steady-state values and the transient dynamics of Tr .

1) *Initial values of N_x and N_{Tr}* : The variable Tr represents an excess-transporters concentration deduced from a reference experiment.

This experiment is characterized by a very low initial concentration of nitrogen: 0.071 g/l which still allows a normal fermentation i. e. a minimum amount of transporters is synthesized so as to degrade all the sugar into ethanol and carbon dioxide.

The ratio N_0/X_{max} (X at steady state) is calculated for this particular experiment, providing the proportion of nitrogen dedicated to biomass growth and synthesis of the minimum amount of transporters needed for an achievable fermentation. This ratio can be applied to the other X_{max} obtained with different N_0 and thus provide the vector $N_{x,0}$, i.e. the initial nitrogen concentration dedicated to biomass growth and synthesis of the minimum amount of transporters:

$$N_{x,0} = \frac{0.071}{X_{max, N_0=0.071}} \cdot X_{max}(N_0), \text{ for } N_0 = 0.071 \rightarrow 0.57 \text{g/l} \quad (4)$$

This is illustrated in the figure 1. The initial nitrogen concentration dedicated to excess-transporters synthesis can be deduced by

$$N_{Tr,0} = N_0 - N_{x,0} \quad (5)$$

as it is depicted with the green curve in figure 1. For $N_0 = 0.071$ g/l, $N_{Tr,0}$ is naturally equal to 0.

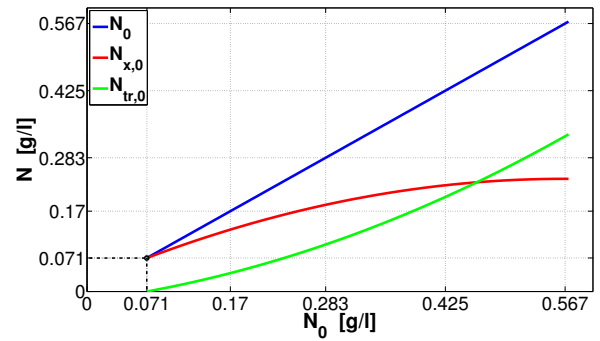


Fig. 1. Initial nitrogen distribution following their use

2) *Steady-state value Tr_{max}* : 16% of nitrogen into proteins is the proportion commonly mentioned in the literature [14]. This ratio can be applied in our case to determine the Tr_{max} (steady-state value) corresponding to $N_{Tr,0}$:

$$Tr_{max} = \frac{N_{Tr,0}(N_0)}{0.16}, \text{ for } N_0 = 0.07 \rightarrow 0.57 \text{g/l} \quad (6)$$

This is illustrated by the blue curve in figure 2. It also implies that the value of the parameter k'_1 is equal to 0.16.

3) *Transient behaviour of Tr* : As transporters are proteins synthesized by yeast, their transient behaviour is considered as the same as the biomass. We consider that

- $Tr_0 = 0$ g/l;
- after being subtracted from the initial value X_0 the biomass measurements are scaled to the corresponding Tr_{max} value.

The parameters related to Tr can be estimated.

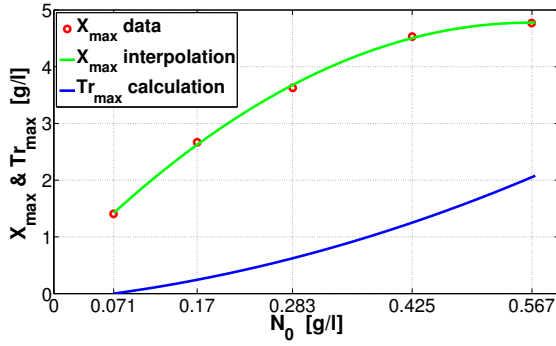


Fig. 2. Steady-state values of X and Tr related to the initial concentration of nitrogen N_0

C. Experimental database

Numerous experiments have been produced to identify the parameters of the Malherbe *et al.* model that are partly described in figure 3. The ranges of temperature and initial amount of nitrogen have been defined large enough to cover even less usual wine-making conditions (high values of nitrogen or temperature). This experimental database has been exploited in the present work: the big black dots correspond to experiments used to estimate the parameters and the small ones to experiments dedicated to cross-validation.

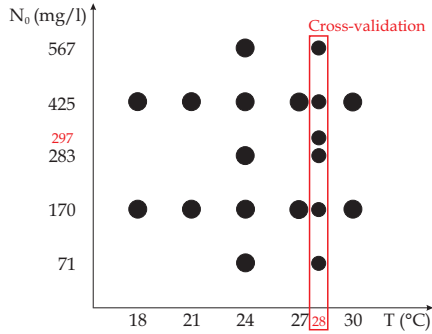


Fig. 3. Experimental planning used to estimate model parameters and validate the model (cross-validation)

D. Model-parameters identification

The model differential equations are solved using the ODE-solvers toolbox from Matlab and the parameters have been estimated by formulating a least-squares problem that could be minimized using dedicated functions like `fminsearch` or `lsqnonlin` (again from Matlab). The model has a cascade structure which makes easier the identification task: the parameters $\mu_{max}(T)$, K_x and k_1 are identified, then $\eta_{max}(T)$, K_{tr} , and finally $\beta_{max,1}(T)$, $\beta_{max,2}(T)$, $K_S(T)$, $K_E(T)$ and $\phi(T)$. Most of the estimated parameters are temperature-dependent and table I shows their range of values related to the range of temperature $[18:30]^\circ\text{C}$.

The values of $\eta_{max}(T)$, K_{tr} and $\phi(T)$ can be just considered as reasonable values face to the other parameters of the model as no source can provide information about the dynamical behaviour of the transporters.

TABLE I
MODEL-PARAMETERS VALUES

Parameter	Range of value	Unit
T	18 – 30	$^\circ\text{C}$
$\mu_{max}(T)$	0.0996 – 0.3034	h^{-1}
K_x	0.0127	-
k_1	0.0528	-
$\eta_{max}(T)$	0.0799 – 0.2257	h^{-1}
K_{tr}	0.3134	g/l
$\beta_{max,1}(T)$	0.1963 – 0.3759	h^{-1}
$\beta_{max,2}(T)$	0.3318 – 0.9205	h^{-1}
$K_S(T)$	7.6560 – 2.9011	g/l
$K_E(T)$	47.7440 – 26.3585	g/l
$\phi(T)$	0.3887 – 0.4727	$(\text{g/l})^{-1}$

E. Model validation

Figure 4 illustrates the evolution of the different variables during a standard fermentation: growth of the biomass, consumption of sugar and production of CO_2 and ethanol, consumption of the nitrogen split into two nitrogen substrates and above all the flow of CO_2 ($\dot{C}\text{O}_2$) representing the fermentation activity. This measurement is really representative of the process and moreover is precise and easy to do. It can be observed that the model provides good predictions of the fermentation through this CO_2 flow even if the peak for experiments at $N_0 = 71$ mg/l is a bit overestimated (see first peaks of $\dot{C}\text{O}_2$ in figure 10).

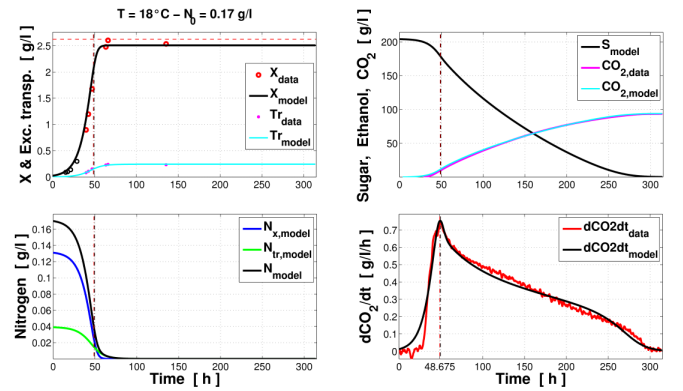


Fig. 4. Resulting curves of the model (*model) face to the experimental curves (*data). The vertical lines correspond to the respective instants when $\text{CO}_2 = \max(\text{CO}_2)$, see last sub-figure

Figure 5 shows the cross-validation results. They are pretty good for the first three graphs but not for the last one which exhibits a limit of the model. Indeed, this experiment is characterized by a large initial amount of nitrogen ($N_0 = 570$ mg/l) and therefore the assumption of nitrogen as limiting substrate is probably no longer valid in this specific case. Indeed, other substrates presumably become limiting like illustrated in [15] explaining that the model provides a too optimistic evolution of the fermentation.

III. FERMENTATIONS WITH NITROGEN ADDITION

A nitrogen addition during the fermentation allows to reboost the fermentation activity (represented by $\dot{C}\text{O}_2(t)$)

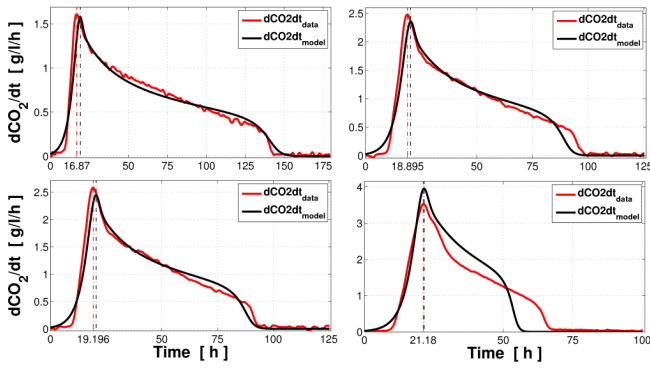


Fig. 5. Resulting curves of CO_2 flow for the model (**model*) face to the experimental curves (**data*), for cross-validation experiments i.e. with temperature equal to 28°C and N_0 equal respectively to 170, 283, 297 and 567 mg/l. The vertical lines correspond to the respective instants when $\dot{C}\text{O}_2 = \max(\dot{C}\text{O}_2)$

so as to shorten the fermentation duration. The extension of the model to the nitrogen additions is consequently of interest for future experiments. The database used to estimate the parameters of the Malherbe *et al.* model [9] includes some experiments with nitrogen additions that are depicted in figures 6 and 7. An experiment at 24°C and $N_0 = 71$ mg/l has been repeated several times, and for each run a nitrogen amount of 63 mg/l has been added at a different stage of the fermentation (the maximum legal amount when experiments were made).

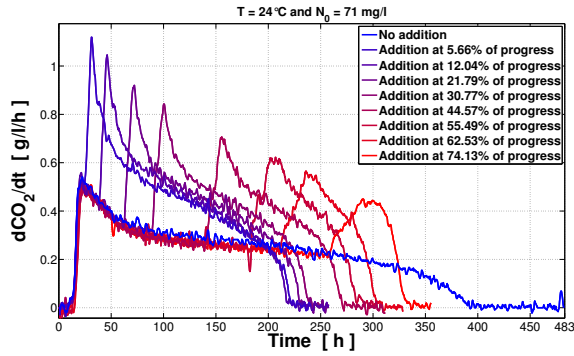


Fig. 6. Measurements of CO_2 flow for experiments with nitrogen addition of 300 mg/l $(\text{NH}_4)_2\text{HPO}_4$ providing 63 mg/l of atomic nitrogen, entirely assimilable

These figures show that when the nitrogen is added in the first half of the fermentation there is a regrowth of biomass explaining the reboot of fermentation activity. Nevertheless if the addition is made in the second part of fermentation, this regrowth is weak and finally tends to zero (addition at 62.53 and 74.13% for instance) whereas the fermentation activity still reaches a peak. This can be explained by the use of nitrogen to synthesize transporters so as to reboot the existing-cells activity instead of using this nitrogen to produce new cells [12]. Therefore the variable Tr can be used to represent this phenomenon and the previous model can be extended.

It can be observed in figure 6 that the span of time between

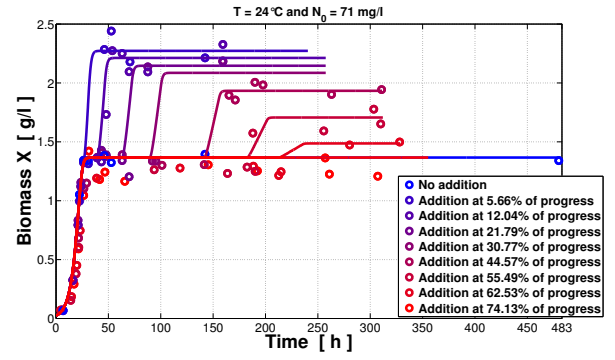


Fig. 7. Measurements of biomass X for experiments with nitrogen addition (colored dots) and resulting curves of the model (colored solid lines)

the instant of nitrogen addition t_{add} and the instant where the second peak of activity is reached tends to be longer as the addition is made at a late stage of fermentation. This evolution of the “rise time” t_{rise} is illustrated in the first graph of figure 8. If there is a nitrogen addition at 0% of progress, t_{rise} is equal to zero. The experiment with the addition at 55.49% of progress has been dropped out as its t_{rise} seems to be an artefact (it is due to measurement noise for the second $\dot{C}\text{O}_2(t)$ peak). An inflection point is deduced from the interpolation curve that will also be observed for most of the new parameters introduced in the modeling extension (figure 8).

A. Model equations

The model-extension formulation depends on the instants t_{add} and $t_{add} + t_{rise}$. When $t < t_{add}$, the fermentation dynamics is predicted by the previous model described in Section II-A.

The impulse of nitrogen issued from the addition is distributed on the time span $[t_{add}, t_{add} + t_{rise}]$ representing the dynamics of the nitrogen use following the stage of fermentation.

Therefore when $t_{add} \leq t \leq t_{add} + t_{rise}$,

$$\begin{cases} \dot{X} = \mu_{max}(T) \frac{N_x}{K_x X + N_x} X & \text{and} & \dot{N}_x = -k_1 \dot{X} + \alpha \frac{N_{add}}{t_{rise}} \\ \dot{Tr} = \eta_{max}(T) \frac{N_{tr}}{K_{tr} + N_{tr}} X & \text{and} & \dot{N}_{tr} = -k'_1 \dot{Tr} + (1 - \alpha) \frac{N_{add}}{t_{rise}} \\ \dot{E} = \dot{C}\text{O}_2 = \left(\beta_{max,1}(T) \frac{N_x}{K_x X + N_x} + \beta_{max,2}(T) \frac{K_E(T)}{K_E(T) + E} \right. \\ \quad \left. + \beta_{max,3} \frac{N_{tr}}{K_{tr} + N_{tr}} \right) \frac{S}{K_S(T) + S} X (1 + \phi_2 Tr) \\ \dot{S} = -k_2 \dot{E} \end{cases}$$

where N_{add} corresponds to the amount of nitrogen that is added, α represents the proportion of nitrogen used for the biomass regrowth, $\beta_{max,3}$ is a new maximum specific reaction rate and ϕ_2 is a new efficiency parameter related to Tr replacing ϕ .

When $t > t_{add} + t_{rise}$,

$$\begin{cases} \dot{X} = \mu_{max}(T) \frac{N_x}{K_x X + N_x} X & \text{and } \dot{N}_x = -k_1 \dot{X} \\ \dot{Tr} = \eta_{max}(T) \frac{N_{Tr}}{K_{Tr} + N_{Tr}} X & \text{and } \dot{N}_{Tr} = -k'_1 \dot{Tr} \\ \dot{E} = \dot{CO}_2 = \left(\beta_{max,1}(T) \frac{N_x}{K_x X + N_x} + \beta_{max,2}(T) \frac{K_E(T)}{K_E(T) + E} \right. \\ \quad \left. + \beta_{max,3} \frac{N_{Tr}}{K_{Tr} + N_{Tr}} \right) \frac{S}{K_S(T) + S} X (1 + \phi_2 Tr) \\ \dot{S} = -k_2 \dot{E} \end{cases}$$

The value of the three parameters α , $\beta_{max,3}$ and ϕ_2 depends on the stage of fermentation at which the nitrogen addition is made and have been estimated with the set of experiments described in figures 6 and 7. They are illustrated in the last three graphs of figure 8.

As expected α varies between 0 and 1. The interpolation curve (in green on second graph of figure 8) has been calculated with weights on each estimated point following the number of available measurements of X describing the re-growth in each experiment. For instance the point corresponding to the experiment with addition at 30.77% of progress had only one measurement whereas other experiments had more than two measurements. The point corresponding to the experiment with addition at 74.13% was not accounted for the interpolation. Indeed, by observing the experiment with addition at 62.53% it is clear that α probably passes to zero if the nitrogen addition is made at a stage of fermentation between 65 and 70%.

The evolution of the parameter ϕ_2 has been interpolated so as to respect the continuity with the model described in Section II-A i.e. the value of ϕ_2 for a nitrogen addition at 0% of progress cannot be inferior to the corresponding value of ϕ .

The last experiment (addition at 74.13%) has not been accounted for to interpolate the estimated values of $\beta_{max,3}$ like for the parameter α .

Inflection points can be observed in figure 8 for t_{rise} , α and ϕ_2 evolving between 20 and 30% of fermentation progress. This could be considered as a switch in the dynamics of the nitrogen consumption (the maximum value of $\beta_{max,3}$ could even be considered in that way given the lack of precision due to the interpolation).

B. Model validation

The validation of this model extension is made with the limited number of experiments used for the new parameters estimation. The figure 9 (addition at 44.57%) illustrates the nitrogen-addition impact on all the variables: the biomass regrows, new transporters are synthesized (first graph) and a new peak of activity ($\dot{CO}_2(t)$) appears slightly underestimated by the model (fourth graph). The nitrogen profile that is applied ($N_{profile}$ function of t_{rise}) is depicted in red in the third graph. The total nitrogen curve is the addition of the curves corresponding to N_x (blue) and N_{Tr} (green).

Figure 10 shows the fermentation activity for other experiments (respectively with addition at 12.04, 30.77, 62.53 and

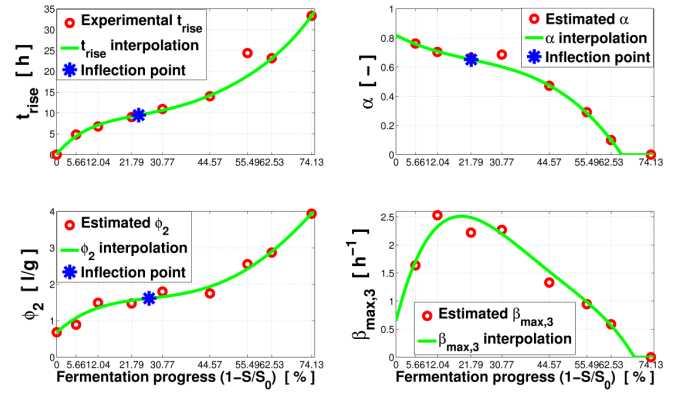


Fig. 8. Evolution of the parameters t_{rise} , α , ϕ_2 and $\beta_{max,3}$

74.13%) and it can be concluded that the model can provide a very good prediction of the fermentation with the early nitrogen additions. The last experiments are well described for X (see simulation curves in figure 7) but the CO_2 flux is underestimated as the addition is late in the fermentation. Nevertheless, the qualitative prediction is still valuable as the peak and the end of fermentation are well described. The

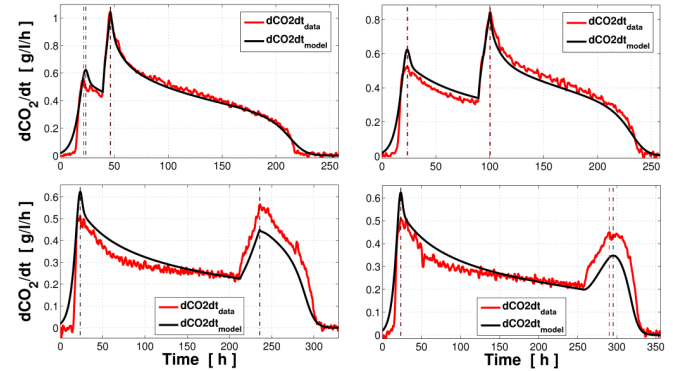


Fig. 10. Resulting curves of CO_2 flow for the model (*model) face to the experimental curves (*data), for the experiments where the nitrogen addition is made respectively at 12.04, 30.77, 62.53 and 74.13% of progress in the fermentation. The vertical lines correspond to the respective instants when $CO_2 = \max(CO_2)$, before and after the nitrogen addition

model finally predicts standard fermentations and moreover fermentations with nitrogen addition whatever the addition instant, which therefore outperforms for instance the model of Malherbe *et al.* [9] that only includes nitrogen addition at a stage of fermentation of around 50%.

IV. CONCLUSIONS AND FUTURE WORK

A. Conclusions

The present comprehensive model of wine-making fermentations includes as a variable Tr , (excess) glucose transporters, proteins that have an important role in the fermentation activity. The use of nitrogen for the growth of biomass and for the synthesis of these specific proteins is described with a set of relatively simple ordinary differential equations that allows to efficiently predict the evolution of a fermentation. The importance of the nitrogen distribution and of Tr

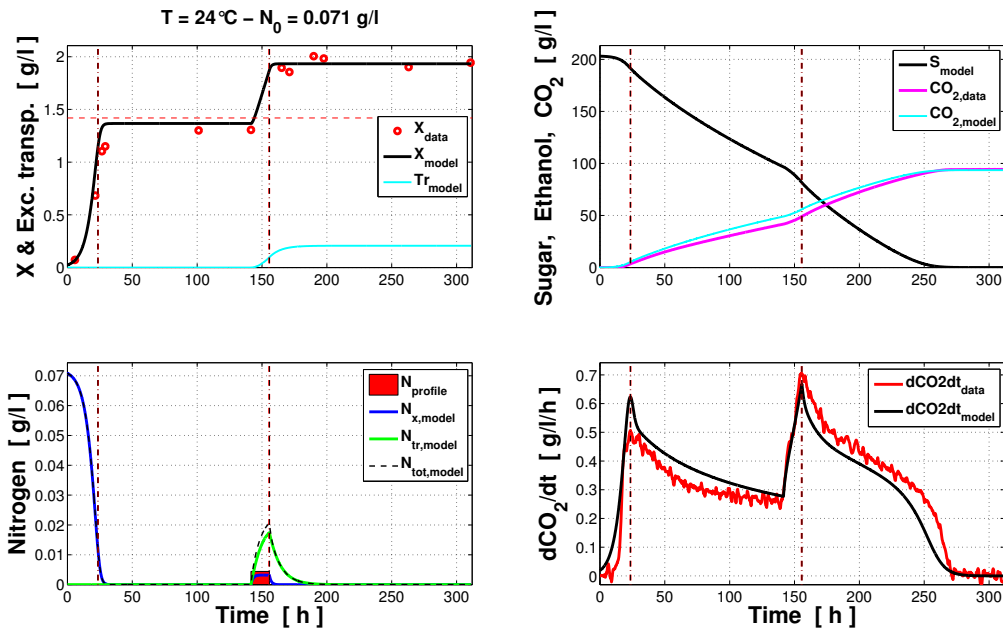


Fig. 9. Resulting curves of the model (*model) face to the experimental curves (*data) for the experiment where the nitrogen addition is made at 44.57% of progress in the fermentation. The vertical lines correspond to the respective instants when $\dot{CO}_2 = \max(\dot{CO}_2)$ before and after the nitrogen addition, see last sub-figure

is reinforced in the model extension allowing the addition of nitrogen during the fermentation. The resulting mass-balance model outperforms one of the more validated must-fermentation models and consequently provides a reliable tool that can be used for control purposes.

B. Future work

The nitrogen-addition phenomena could be more largely studied thanks to similar experiments (different N_0 or N_{add}). The whole flavour aspect of the wine-making will also be explored in order to extend the present model.

V. ACKNOWLEDGMENTS

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