HAL open science

Modelling the effects of assimilable nitrogen addition on fermentation in oenological conditions

François Beaudeau, César Arturo Aceves-Lara, Josephine Godilllot, Jean-Roch Mouret, Ioan-Cristian Trelea, Carine Bideaux

To cite this version:

François Beaudeau, César Arturo Aceves-Lara, Josephine Godilllot, Jean-Roch Mouret, Ioan-Cristian Trelea, et al.. Modelling the effects of assimilable nitrogen addition on fermentation in oenological conditions. Bioprocess and Biosystems Engineering, 2023, 46, pp.941-955. 10.1007/s00449-023-02861-w . hal-04102855

HAL Id: hal-04102855

https://agroparistech.hal.science/hal-04102855

Submitted on 22 May 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Modeling the Effects of Assimilable Nitrogen Addition on Fermentation in Oenological Conditions

François Beaudeau[1,2], Cesar Arturo Aceves Lara[1,3], Josephine Godilllot[4,5], Jean-Roch Mouret[5,6], Ioan-Cristian Trelea[7,8], Carine Bideaux[1,9]

Abstract

Alcoholic fermentation in oenological conditions is a biological process carried out under significant physiological constraints: deficiency of nitrogen and others nutriments (vitamins, lipids …) and different stresses (pH and osmotic). In literature, few models were proposed to describe oenological fermentations. They are focused on initial conditions and did not integrate nitrogen addition during the fermentation process which is a widespread practice. In this work, two dynamic models of oenological fermentation are proposed to predict the effects of nitrogen addition at two different timings: at the beginning of the process and during the fermentation experiment. They were validated and compared against existing models showing an accurately fit to experimental data for CO_2 release and CO_2 production rate.

Keywords: mathematical modeling, oenological fermentation, nitrogen addition

Data availability statement

The datasets presented un this study can be found in online repositories. The names of the repository/repertories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo., GSE189010.

Funding Statement

This research project was funded by the French National Research Agency (ANR) under grant agreement STARWINE (ANR-18-CE10-0013).

[1] TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France
[2] beaudeau@insa-toulouse.fr
[3] aceves@insa-toulouse.fr
[4] SPO, INRAE, L'institut Agro Montpellier, Université de Montpellier, Montpellier, France
[5] josephine.godillot@inrae.fr
[6] Jean-roch.mouret@inrae.fr
[7] Université Paris-Saclay, INRAE, AgroParisTech, UMR Sayfood, F-78850, Thiverval-Grignon, France
[8] Ioan-cristian.trelea@inrae.fr
[9] bideaux@insa-toulouse.fr

Nomenclature

State variables:

- X Biomass in 10^9cells/L
- S Sugar in g/L
- E Ethanol in g/L
- CO_2 CO_2 produced in g/L
- N_{ST} Sugar transporter in $g_N/(10^9\text{cells})$
- N Nitrogen in g/L
- N_{intra} Intracellular nitrogen in $g/(10^9\text{cells})$
- A Cell activity (dimensionless)

Rates:

- r_C rate of production or consumption of product C (X: biomass, S: sugar, N: nitrogen, E: ethanol, CO_2: carbon dioxide, N_{intra}:intracellular nitrogen and N_{ST}: sugar transporter) in g/(L.h).
- r_i^* rate of equation i in the model 2 in mol/(L.h).

Molar masses:

- M_S molar mass of sugar in g_S/mol_S
- M_E molar mass of ethanol in g_E/mol_E
- M_X biomass mass per carbon mole in $g_X/Cmol_X$
- M_{CO2} molar mass of carbon dioxyde in g_{CO2}/mol_{CO2}
- M_N molar mass of nitrogen in g_N/mol_N

Model 1 parameters:

- k_1 maximum specific growth rate in h^{-1}
- k_2 maximum specific rate of sugar consumption in $(10^9\text{cell. } g_N.h)^{-1}$
- K_S affinity constant in g_S/L
- K_{SI} inhibition constant in $(L/g_E)^{\alpha S}$
- α_S exponent of ethanol inhibition of sugar consumption, dimensionless
- $Y_{E/S}$ conversion yield of sugar to ethanol in g_S/g_E
- $Y_{CO2/S}$ conversion yield of sugar to CO_2 in g_S/g_{CO2}
- k_3 maximum specific rate of nitrogen consumption in $(10^9\text{cell.h})^{-1}$
- K_N affinity constant in g_N/L
- K_{NI} inhibition constant in $(L/g_E)^{\alpha N}$
- α_N exponent of ethanol inhibition of nitrogen consumption, dimensionless
- Q_0 minimum intracellular nitrogen concentration for biomass production in $g_N/(10^9\text{cell})$
- E_{max} critical ethanol concentration in g_E/L
- k_{nst} maximum specific production rate of sugar transporter in $g_N/(10^9\text{cell.h})$
- k_{dnst} maximum specific degradation rate of sugar transporter in $g_N/(10^9\text{cell.h})$
- K_{nst} affinity constant of sugar transporter in $g_N/(10^9\text{cell})$
- Y_{nst} nitrogen yield of sugar transporter in g_N/g_N
- Q_{0nst} minimal intracellular nitrogen content for sugar transporter production in $g_N/(10^9\text{cell})$
- α_1 nitrogen yield in cell in $g_N/(10^9\text{cell})$
- κ cell activity loss rate in h^{-1}

Model 2 parameters:

- k_X maximum specific growth rate $(Cmol_X.h)^{-1}$
- N_{intra0} constant of growth limitation by intracellular nitrogen in $g_N/(10^9\text{cell})$
- K_{S1} affinity constant for sugar in g_S/L
- k_E maximum specific ethanol production rate in $mol_E/(Cmol_X.h)$
- K_{S2} affinity constant for sugar in g_S/L
- E_{lim1} constant of ethanol production inhibition by ethanol in g_E/L
- α_1 exponent of ethanol inhibition of ethanol production, dimensionless
- K_{st} sugar transporter limitation constant in g_N/L

- k_N maximum specific nitrogen consumption rate $mol_N/(Cmol_X.h)$
- K_{N1} affinity constant for nitrogen in g_N/L
- g_1 number of moles of sugar consumed per Cmole of biomass produced in $mol_S/Cmol_X$
- g_2 number of moles of nitrogen consumed per Cmole of biomass produced in $mol_N/Cmol_X$
- g_3 number of moles of CO_2 produced per Cmole of biomass produced in $mol_{CO2}/Cmol_X$
- k_{NST} maximum specific sugar transporter production rate in $mol_N/(L.h)$
- E_{lim2} constant of sugar transporter production inhibition by ethanol in g_E/L
- α_2 exponent of ethanol inhibition of sugar transporteur production, dimensionless
- N_{intra1} constant of sugar transporter limitation by intracellular nitrogen in $g_N/(10^9 cell)$
- κ cell activity loss rate in h^{-1}
- g_S conversion yield of sugar into ethanol in mol_E/mol_S
- a number of cells per biomass gram in $10^9 cell/g$

1 Introduction

Oenological fermentation is a well-known biological process in which yeasts, mainly *Saccharomyces cerevisiae* strains, convert hexoses (glucose and fructose) principally to ethanol and carbon dioxide, secondarily to biomass and glycerol and weakly to metabolites produced in low concentrations with major impact on wines organoleptic properties. Oenological fermentation is a process which has several limitations whose main one is deficiency in nitrogen. In industrial conditions, nitrogen is totally consumed in the first two days of fermentation whose "normal" duration is one to two weeks [1]. However nitrogen is an essential nutrient for yeast metabolism and growth during oenological fermentation [2, 3] and its deficiency can induce sluggish or stuck fermentations [4]. Several studies have been conducted to understand and quantify nitrogen deficiency: Bisson [5] related nitrogen deficiency to stuck and sluggish fermentations, Crépin et al [6] studied the sequential uptake of nitrogen sources by yeasts and Carrau et al [7] studies the impact of nitrogen deficiency on aroma compounds. Nitrogen addition during fermentation increases reaction kinetics but this increase depends on addition timing [8]. That is why European legislation allows nitrogen addition at the beginning and during wine oenological fermentation [9].

David et al [10] shows that residual growth is observed after the total consumption of nitrogen present initially in the medium which would suggest that the yeasts have a nitrogen reserve to continue their growth. Brou et al [11] shows that intracellular nitrogen concentration decreases to a minimum during fermentation. It is therefore important to consider the intracellular nitrogen concentration in the yeasts.

The data from David et al [10] shows also that a regrowth of biomass is possible after nitrogen addition and that this regrowth is all the stronger the earlier the nitrogen is added. Indeed, yeast physiological state declines during fermentation due to several deficiency and inhibitions such as inhibition by ethanol. Thus, the increase of ethanol concentration during fermentation affects physiological state of yeast cells: activities of nitrogen transporters [12, 13] and sugar transporters [14] are decreasing when the ethanol concentration increases. Moreover, a study shows a rapid decrease of cell activity after ethanol addition in the medium [15].

Several mathematical models were proposed to simulate fermentation in oenological conditions [16-22]. They consider time evolutions of concentrations for biomass, sugar, ethanol, carbon dioxide and some of them for nitrogen and glycerol but few models consider the relation between yeast growth and nitrogen concentration:

- Cramer's model [23] considers nitrogen limitation in the biomass growth with a Monod-type equation and a non-growth associated conversion of glucose into ethanol. It considers too a cell death rate proportional to ethanol concentration.
- Malherbe's model [8] describes the biomass production as a logistic law with a maximum attainable biomass concentration dependent on the initial nitrogen concentration. The authors introduce in their model a state variable representing sugar transporters involved in the sugar consumption rate equation. Moreover, the ethanol inhibition is considered in the equations of sugar and nitrogen consumption rates. Ethanol and CO_2 are produced from sugar in an overall yield (1 gram of sugar is converted into 2.17 grams of ethanol and 2.17 grams of CO_2).
- Brou's model [11] is based on an intracellular nitrogen compartment divided between the minimum nitrogen necessary to carry out the vital functions of the cells and a storage compartment, which is the only nitrogen available for biomass growth. It considers ethanol inhibition on metabolism of yeasts (production of biomass, ethanol and glycerol) as well as the cell death rate function of ethanol concentration.

However, these models were not developed nor calibrated for fermentations with nitrogen addition during the fermentation process. Cramer's and Brou's models can allow regrowth of biomass after nitrogen add. Malherbe's model could simulate nitrogen addition with a complicated algorithm: model is simulated using initial conditions and it is stopped before nitrogen addition instant and new simulation is started with the new initial conditions. This is a difficult task if we try to apply the model for online optimization purposes.

In this work, two models derived from Malherbe and Brou's models are proposed to allow the simulation of nitrogen addition during fermentation without stopping the simulation at nitrogen addition instant (section 3). These models have been calibrated on experimental data from fermentations with different initial nitrogen concentrations and different nitrogen amount addition at an add timing corresponding to 20g/L of CO_2 release allowing biomass regrowth (section 4). A sensitivity analysis of the two models is presented in section 5. Then, the two models have been validated and compared to Malherbe's and Brou's models on experimental data from others fermentations with various initial nitrogen conditions and nitrogen addition at 20 and 35g/L produced CO_2 (section 6).

2 Materials and Methods

2.1 Yeast strain

The fermentations, which are used in this study, are performed with the commercial strain of *Saccharomyces cerevisiae* Lalvin EC-1118 (Lallemand SA, Montreal, Canada). Fermentation tanks are inoculated with 100 mg/L active dry yeast, i. e. 10^9 cells/L, which are rehydrated for 30 minutes at 37°C in a 50 g/L glucose solution.

2.2 Fermentation media

The fermentations are carried out in synthetic musts exhibiting the characteristics of a standard grape juice [24]. The sugar concentration of all musts is 180g/L evenly split between glucose and fructose and the pH is at 3.3. Three initial concentrations of assimilable nitrogen (ammonium chloride and mixture of amino acids) are used: 70, 140 and 210 mg_N/L, corresponding respectively to MS70, MS140 and MS210. The three media present the same proportions of the different nitrogen sources [25]. The concentrations of others nutrients are the same as in [25].

2.3 Fermentation conditions

The fermentation are carried out in 1.2 L glass fermenters with 1 L of medium regulated at 24°C. CO_2 release is accurately measured from an automatic online monitoring of weight loss [26].
Nitrogen addition is carried out with three concentrations: 50, 100 and 150 mg_N/L in the form of mineral nitrogen (di-ammonium phosphate) and different timings: at 20 g/L produced CO_2 for calibration fermentations and at 20 g/L and 35g/L produced CO_2 for validation fermentations. Nitrogen additions are performed automatically by means of a pump [27].

2.4 Cell population

The total cell population is measured using a Beckman Coulter counter (Model Z2, Beckman-Coulter, Margency, France) fitted with a 100 µm aperture probe.

2.5 Measurement of Assimilable Nitrogen

Ammonium concentration is measured enzymatically (R-Biopharm, Darmstadt, Germany). The free amino acid concentrations are measured by cation exchange chromatography with post-column ninhydrin derivatization (Biochrom 30, Biochrom, Cambridge, United Kingdom) as presented by Crépin et al [6] .

2.6 Fermentation conditions of data used for models' calibration and validation

The conditions of the cultures used for the calibration and the validation of the models are presented in Table 1.

Table 1 Experimental conditions of calibration fermentations

Fermentations		Initial nitrogen (mg/L)	Added nitrogen (mg/L)	CO_2 produced at nitrogen addition (g/L)	time at nitrogen addition (h)
Calibration	MS70+100	70	100	20	71.412
	MS140+50	140	50	20	37.51
	MS140+150	140	150	20	37.52
	MS210+100	210	100	20	33.72
Validation	MS70+100	70	100	20	67.2
	MS140+50	140	50	20	34.94
	MS140+150	140	150	20	35.97
	MS210+100	210	100	20	29.85
	MS140+100	140	100	35	57.88

3 Models formulation

Two structured models were developed based on nitrogen-limited biomass growth to simulate nitrogen addition without discontinuities, one based on Malherbe's model and another on Brou's model. These new models allow to simulate the fermentation variables: biomass (X in 10^9cell/L), sugar (S in g/L), nitrogen (N in g/L) and ethanol (E in g/L), the cumulative production of carbon dioxide (CO_2 in g/L) and also the dynamic of state variables representative of yeast physiological state which are intracellular nitrogen (N_{intra} in g/10^9cell), sugar transporter (N_{ST} in g_N/10^9cell) and cell activity (A dimensionless).

3.1 Introduction of intracellular nitrogen and cell activity

As reported in the literature, a residual growth is observed after the end of nitrogen consumption present in the medium [10]. So, as in the Brou's model, an intracellular nitrogen compartment (N_{intra}) is considered in the models. The intracellular nitrogen is formed by entry of nitrogen into the yeast and is used for biomass growth and sugar carrier production as shown in the **Erreur ! Source du renvoi introuvable.**. As in the Malherbe's model, the sugar transporters (N_{ST}) are considered. They are synthetized from intracellular nitrogen. The sugar transporters are involved in the sugar equation and depict the sugar affinity and the ethanol inhibition of sugar transporter on sugar consumption.

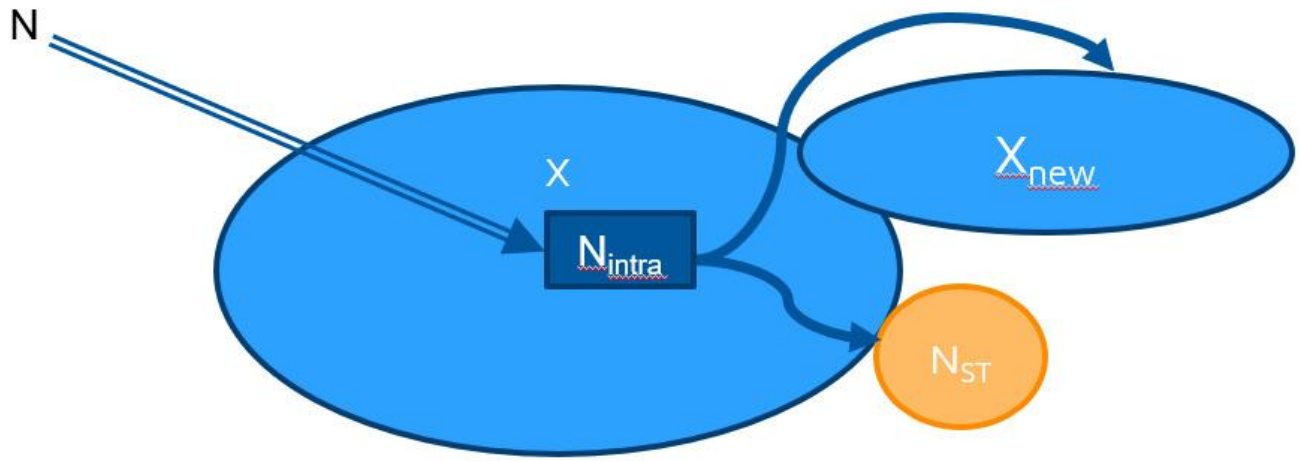

Figure 1 Diagram of nitrogen forms

As it is also known that the physiological state of yeasts declines during the fermentation [12-15] , the term of cell activity was introduced in the models to consider enzymatic activity decrease due to nitrogen deficiency and ethanol production. Its evolution is described in equation (1).

$$\frac{dA}{dt} = \mu\,(1 - A) - \kappa\, A \tag{1}$$

μ (h^{-1}) is the specific growth rate and κ (h^{-1}) a constant.

3.2 Mass balance

The bioreactor is assumed to be infinitely mixed with a constant volume without any transfer limitation. Then the temporal evolution of variables is only linked to the biological kinetics. Based on these assumptions, unsteady-state mass balances for cells, sugar, nitrogen, ethanol, CO_2, intracellular nitrogen and sugar transporter are given by Eqs (2)-(8):

$$\frac{dX}{dt} = r_X \tag{2}$$

$$\frac{dS}{dt} = r_S \tag{3}$$

$$\frac{dN}{dt} = r_N \tag{4}$$

$$\frac{dE}{dt} = r_E \tag{5}$$

$$\frac{dCO_2}{dt} = r_{CO2} \tag{6}$$

$$\frac{dN_{intra}}{dt} = r_{N_{intra}} - \frac{N_{intra}}{X}\frac{dX}{dt} \tag{7}$$

$$\frac{dN_{ST}}{dt} = r_{N_{ST}} - \frac{N_{ST}}{X}\frac{dX}{dt} \tag{8}$$

With r_X, r_E and r_{CO2} the production rate of respectively biomass, ethanol and CO_2 and r_S and r_N the consumption rate respectively of sugar and nitrogen, r_{Nintra} and r_{NST} the net rate of respectively N_{intra} N_{ST}. The term $-\frac{N_{ST}}{X}\frac{dX}{dt}$ and $-\frac{N_{intra}}{X}\frac{dX}{dt}$ are the term of dilution of respectively sugar transporter and intracellular nitrogen due to biomass growth.

3.3 Model 1 derived from Malherbe's model

Malherbe's model [8] has to be modified to integrate effect of nitrogen addition and potential regrowth of biomass after nitrogen addition as biomass depends only on initial nitrogen in this model. Model 1 includes 18 parameters against 16 for the Malherbe's model.

The sugar consumption rate [8] is expressed by:

$$r_S = -\nu_{ST}(S,E)N_{ST}\mathrm{X} \tag{9}$$

In which $\nu_{ST}(S,E)$ represent the activity of a sugar transporter (facilitated mechanism diffusion) with ethanol inhibition:

$$\nu_{ST}(S,\mathrm{E}) = \frac{k_2 S}{S + K_s + K_{S1}SE^{\alpha_S}} \tag{10}$$

With k_2 the maximum specific rate in $g_S/(g_N.10^9$cells.h), K_S the affinity constant in g_S/L, K_{SI} the inhibition constant in $(L/g_E)^{\alpha S}$ and α_S dimensionless.

Assuming a constant yield of CO_2 released and ethanol produced during fermentation, the ethanol and CO_2 productions rates are described by Eq(11) and (12):

$$r_E = -\frac{1}{Y_{\frac{E}{S}}} r_S \tag{11}$$

$$r_{CO_2} = -\frac{1}{Y_{\frac{CO2}{S}}} r_S \tag{12}$$

$Y_{E/S}$ and $Y_{CO2/S}$ are the conversion yields respectively of sugar to ethanol and of sugar to CO_2. they are set to 2.17 [8].
It is assumed that the sugar transporter synthesis depends on intracellular nitrogen and that sugar transporters can be degraded. The net rate of sugar transporter is defined by:

$$r_{N_{ST}} = k_{nst}\left(1 - \frac{Q_{0nst}}{N_{intra}}\right) - k_{dnst}\frac{N_{ST}}{K_{NST} + N_{ST}} \tag{13}$$

In which k_{nst} is the maximum specific production rate in $g_N/(10^9$cell.h), Q_{0nsst} is the minimal intracellular nitrogen content for sugar transporter production in $g_N/(10^9$cell), K_{NST} is the affinity constant in $g_N/10^9$cell and k_{dnst} is the maximum specific degradation rate in $g_N/(10^9$cell.h).

The specific rate of nitrogen consumption of Malherbe's model is modified by introducing the notion of cell activity:

$$r_N = -\mu_N(N,E).X.A \tag{14}$$

μ_N ($g_N/(10^9$ cell.h) correspond to specific nitrogen consumption rate which is limited by nitrogen and inhibited by ethanol. It is defined by:

$$\mu_N(N,E) = \frac{k_3 N}{N + K_N + K_{NI} N E^{\alpha_N}} \tag{15}$$

With k_3 the maximum specific consumption rate in $g_N/(10^9 cell.h^{-1})$, K_N the affinity constant in g_N/L, K_{NI} the inhibition constant and α_N dimensionless.

For the net production rate of intracellular nitrogen (eq 16). Three phenomena are considered: the consumption of nitrogen by yeast cell, the biomass production and the production and degradation of sugar transporters.

$$r_{N_{intra}} = \mu_N(N,E) - \alpha_1 r_X - \frac{1}{Y_{nst}}\left(k_{nst}\left(1 - \frac{Q_{0nst}}{N_{intra}}\right) - k_{dnst}\right) \tag{16}$$

With α_1 the nitrogen yield in cell in $g_N/(10^9 cell)$, Y_{nst} the nitrogen yield of sugar transporter in g_N/g_N.

The biomass production rate (eq 17) is function of intracellular nitrogen concentration and cell activity to consider, respectively, the residual growth after nitrogen depletion in the medium and the lower capacity of the yeast to regrow after nitrogen addition for a longer period of nitrogen depletion. It considers also a linear inhibition by ethanol:

$$r_X = k_1(T) X \left(1 - \frac{Q_0}{N_{intra}}\right)\left(1 - \frac{E}{E_{max}}\right) A \tag{17}$$

In which $k_1(T)$ is the maximum specific growth rate depending on temperature (h^{-1}): $k_1(T) = \alpha_{k1} T - \beta_{k1}$, that relation comes from the Malherbe model [8], Q_0 the minimum intracellular nitrogen content of biomass for biomass growth in $g_N/(10^9 cell)$ and E_{max} the critical ethanol concentration for biomass growth in g_E/L.

3.4 Model 2 derived from Brou's model

3.4.1 Stoichiometric equations

Model 2 is based on four stoichiometric reactions (expressed in mol) that represent the major phenomena considered in this model of oenological fermentation *i.e.* biomass production (r_1^*), ethanol production (r_2^*), nitrogen consumption (r_3^*) and sugar transporter production (r_4^*):

- (r_1^*) $g_1 C_6H_{12}O_6 + g_2 N_{intra} \rightarrow CH_{1.741}O_{0.632}N_{0.097} + g_3 CO_2 + g_4 H_2O$
- (r_2^*) $C_6H_{12}O_6 \rightarrow g_S\, C_2H_6O + g_S\, CO_2$
- (r_3^*) $N \rightarrow N_{intra}$
- (r_4^*) $N_{intra} \rightarrow N_{ST}$

Reaction rates r_i^* are in mol/(L.h). The stoichiometric coefficients g_3 and g_4 can be deduced from g_1 and g_2. g_S is the yield of conversion of sugar into ethanol and CO_2. g_S is slightly lower than 2 mol/mol, the theoretical yield of ethanol production, because sugar is also converted into other metabolites like glycerol and aromas. The biomass composition is taken from [11].

3.4.2 Model equations

The model 2 includes 19 parameters against 18 for the Brou's model. The rates of consumption or production of each species can be expressed as a weighted sum of the four stoichiometric equation rates (eq 18 to 24). All these rates are expressed in g/(L.h) except for biomass whose rate is expressed in 10^9 cell/(L.h) considering the molar mass of each species:

- Sugar:

$$r_S = -(r_2^* + g_1 r_1^*)\, M_S \tag{18}$$

- Ethanol:

$$r_E = g_S\, r_2^*\, M_E \tag{19}$$

- CO_2:

$$r_{CO_2} = (g_S\, r_2^* + g_3 r_1^*)\, M_{CO2} \tag{20}$$

- Biomass:

$$r_X = r_1^* a\, M_X \tag{21}$$

- Nitrogen:

$$r_N = -\, r_3^* M_N \tag{22}$$

- Intracellular nitrogen:

$$r_{N_{intra}} = (r_3^* - g_2 r_1^* - r_4^*) M_N \tag{23}$$

- Sugar transporter :

$$r_{N_{ST}} = r_4^* M_N \tag{24}$$

With M_X, M_S, M_E, M_{CO2} and M_N the molar mass, respectively, of biomass, sugar, ethanol, CO_2 and nitrogen in g/mol and a the number of cells per biomass gram in 10^9cell/g.

3.4.3 Reactions rates

We define X_n the biomass concentration in C-mol$_X$/L by the equation:

$$X_n = \frac{X}{aM_X} \tag{25}$$

with X the biomass concentration in 10^9cell/L. The value of a and M_X are those of Brou's model [11]: 37.6 10^9cell/g and 25.0689g/C-mol respectively.

First stoichiometric equation rate (Yeast growth rate (r_1^*)) is limited by intracellular nitrogen (N_{intra}) and by sugar (S). It depends of biomass (X_n) and cell activity (A) and can be expressed by the equation 26:

$$r_1^* = k_X X_n \left(\frac{N_{intra}}{N_{intra} + N_{intra0}}\right)\left(\frac{S}{K_{S1} + S}\right) A \tag{26}$$

With k_X the maximum specific growth rate in h^{-1}, K_{S1} the affinity constant for substrate in g_S/L and N_{intra0} the constant of growth limitation by intracellular nitrogen in g_N/L.
Ethanol production rate (r_2^*) is inhibited by ethanol (E) and is limited by sugar (S) and sugar transporter (N_{ST}):

$$r_2^* = k_E X_n \left(\frac{S}{S + K_{S2}}\right)\left(1 - \frac{E}{E_{lim1}}\right)^{\alpha_1}\left(\frac{N_{ST}}{N_{ST} + K_{ST}}\right) \tag{27}$$

With k_E the maximum specific ethanol production rate in $mol_E.mol_X.h^{-1}$, K_{S2} the affinity constant for sugar in g_S/L, E_{lim1} the critical concentration of ethanol in g_E/L for which there is no more ethanol production and K_{ST} the constant of ethanol production limitation by sugar transporter in $g_N/10^9$cell.

Nitrogen absorption rate (r_3^*) is limited by nitrogen (N) and depends of biomass (X_n) and cell activity (A):

$$r_3^* = k_N X_n \left(\frac{N}{N + K_{N1}}\right) A \tag{28}$$

With k_N the maximum specific nitrogen absorption rate in $mol_N.mol_X.h^{-1}$, K_N the affinity constant for nitrogen in g_N/L.
Sugar transporter rate (r_4^*) is inhibited by ethanol (E) and limited by intracellular nitrogen (N_{intra}):

$$r_4^* = k_{NST} X_n \left(1 - \frac{E}{E_{lim2}}\right)^{\alpha_2}\left(\frac{N_{intra}}{N_{intra} + N_{intra1}}\right) \tag{29}$$

With k_{NST} the maximum specific sugar transporter rate in $mol_N.mol_X.h^{-1}$, E_{lim2} the critical concentration of ethanol in g_E/L and N_{intra1} the constant of sugar transporter limitation by intracellular nitrogen.

Equations of the two models developed are summarized in Table 2.

Table 2 Two models equations

Variable	Model 1	Model 2
Biomass	$\frac{dX}{dt} = k_1(T)X\left(1-\frac{Q_0}{N_{intra}}\right)\left(1-\frac{E}{E_{max}}\right)A$ With $k_1(T) = \alpha_{k1}T - \beta_{k1}$	$\frac{dX}{dt} = k_X X_n\left(\frac{N_{intra}}{N_{intra}+N_{intra0}}\right)\left(\frac{S}{K_{S1}+S}\right)Aa\,M_X$
Sugar	$\frac{dS}{dt} = -\nu_{ST}(S,E)N_{ST}\mathrm{X}$ With $\nu_{ST}(S,\mathrm{E}) = \frac{k_2 S}{S+K_s+K_{S1}SE^{\alpha_S}}$	$\frac{dS}{dt} = -\left(k_E X_n\left(\frac{S}{S+K_{S2}}\right)\left(1-\frac{E}{E_{lim1}}\right)^{\alpha_1}\left(\frac{N_{ST}}{N_{ST}+K_{ST}}\right) + g_1 k_X X_n\left(\frac{N_{intra}}{N_{intra}+N_{intra0}}\right)\left(\frac{S}{K_{S1}+S}\right)A\right)M_S$
Ethanol	$\frac{dE}{dt} = -\frac{1}{Y_{E/S}}\frac{dS}{dt}$	$\frac{dE}{dt} = g_S\,k_E X_n\left(\frac{S}{S+K_{S2}}\right)\left(1-\frac{E}{E_{lim1}}\right)^{\alpha_1}\left(\frac{N_{ST}}{N_{ST}+K_{ST}}\right)M_E$
CO_2	$\frac{dCO_2}{dt} = -\frac{1}{Y_{CO2/S}}\frac{dS}{dt}$	$\frac{dCO_2}{dt} = \left(g_S\,k_E X_n\left(\frac{S}{S+K_{S2}}\right)\left(1-\frac{E}{E_{lim1}}\right)^{\alpha_1}\left(\frac{N_{ST}}{N_{ST}+K_{ST}}\right) + g_3 k_X X_n\left(\frac{N_{intra}}{N_{intra}+N_{intra0}}\right)\left(\frac{S}{K_{S1}+S}\right)A\right)M_{CO2}$
Nitrogen	$\frac{dN}{dt} = -X\mu_N(N,E)A$ With $\mu_N(N,E) = \frac{k_3 N}{N+K_N+K_{NI}NE^{\alpha_N}}$	$\frac{dN}{dt} = -k_N X_n\left(\frac{N}{N+K_{N1}}\right)AM_N$
Intracellular nitrogen	$\frac{dN_{intra}}{dt} = \mu_N(N,E) - \left(\frac{\alpha_1}{X}+\frac{N_{intra}}{X}\right)\frac{dX}{dt} - \frac{1}{Y_{nst}}\left(k_{nst}\left(1-\frac{Q_{0nst}}{N_{intra}}\right)-k_{dnst}\right)$	$\frac{dN_{intra}}{dt} = \left(k_N X_n\left(\frac{N}{N+K_{N1}}\right)A - g_2 k_X X_n\left(\frac{N_{intra}}{N_{intra}+N_{intra0}}\right)\left(\frac{S}{K_{S1}+S}\right)A - k_{NST}X_n\left(1-\frac{E}{E_{lim2}}\right)^{\alpha_2}\left(\frac{N_{intra}}{N_{intra}+N_{intra1}}\right)\right)M_N$
Sugar transporter	$\frac{dN_{ST}}{dt} = k_{nst}\left(1-\frac{Q_{0nst}}{N_{intra}}\right) - k_{dnst}\frac{N_{ST}}{K_{NST}+N_{ST}} - \frac{N_{ST}}{X}\frac{dX}{dt}$	$\frac{dN_{ST}}{dt} = k_{NST}X_n\left(1-\frac{E}{E_{lim2}}\right)^{\alpha_2}\left(\frac{N_{intra}}{N_{intra}+N_{intra1}}\right)M_N$
Cell activity	$\frac{dA}{dt} = \frac{1}{X}\frac{dX}{dt}(1-A) - \kappa A$	$\frac{dA}{dt} = \frac{1}{X}\frac{dX}{dt}(1-A) - \kappa A$

3.6 Computing

Matlab R2017b software was used for calibration and simulation of the models: ode15s function [28] to solve differential equations and pattern search and Rosenbrock algorithms to calibrated models.

3.7 Statistical analysis

Models quality was analysed with experimental data using the Normed Root Mean Square Error (NMRSE) [29] and the Akaike Information Criterion (AIC) [30]. The NRMSE is defined as:

$$NRMSE = \frac{\sqrt{\frac{\sum_{i=1}^{T}(x_i - y_i)^2}{n}}}{y_{max} - y_{min}} \tag{30}$$

With n the number of experimental data, x_i the experimental data corresponding to time t_i, y_i the data simulated by model for the same time t_i, y_{min} and y_{max} the minimum and maximum data values.

the NRMSE is used for several variables, with the following formula:

$$NRMSE_{tot} = \frac{\sum_{i=1}^{m} NRMSE_i \sqrt{n_i}}{\sqrt{\sum_{i=1}^{m} n_i}} \tag{31}$$

m is the total number of variables, $NRMSE_i$ are the NRMSE value for the ith variable and n_i is the number of values of the ith variable.

The AIC is defined by:

$$AIC = 2p + n(ln(2\pi) + ln(SSE) - ln(n) + 1\ ) \tag{32}$$

With p the number of parameters, n the n number of experimental data and SSE the Sum Square Error.

The SSE is defined by:

$$SSE = \sum_{i=1}^{n}(x_i - y_i)^2 \tag{33}$$

With n the number of experimental data, x_i the experimental data corresponding to time t_i, y_i the data simulated by model for the same time t_i..

3.8 Sensibility analysis

The robustness of the models 1 and 2 to its parameters has been studied using the sensitivity coefficient $\sigma_y^{\Delta p}$ (Eq 32) for the variable y and the parameter p [31].

$$\sigma_y^{\Delta p} = \int_0^{t_f} \frac{y(p + \Delta p, t) - y(p, t)}{y(p, t)} dt \tag{34}$$

With t_f the final fermentation time, y(p,t) the simulated value of the variable y at the time t and for the vector of optimized parameters and Δp the modification of a parameter.

4 Models calibration

4.1 Parameters calibration

The two models are calibrated using the four fermentations presented in Table 1 of paragraph 2.6. Each fermentation present between 10 and 18 data of nitrogen and of biomass and about 460 data of CO_2 (one data each 20 minutes).

The Table 3 shows Model 1 and model 2 calibrated parameters, and the confidence intervals for these parameters. It could be seen that calibrated Model 1 have values close to those reported in the literature [8].

The confidence intervals are small for almost all parameters of both models, which means that the parameters values are reliable. For the Model 1, only three parameters (K_S, k_{nst} and K_{nst}) present a great confidence interval compared to their values. For the Model 2, only the parameter α_2 present a great confidence interval compared to its value.

Table 3 Model 1 and 2 and Malherbe's model parameters

Parameters	Model 1	Malherbe's model [8]		Parameters	Model 2
k_1	$\alpha_{k1}T - \beta_{k1}$	$\alpha_{k1}T - \beta_{k1}$		k_X	0.4036±0.0113
α_{k1}	0.0287	0.0287		N_{intra0}	(9.059±0.0000).E-6
β_{k1}	0.3	0.3762		KS_1	1.565±0.0358
k_2	0.0386±0.00038	0.035		k_E	0.2215±0.0047
K_S	20.67±18.3341	15		KS_2	1.955±0.0624
K_{SI}	0.006299±0.00087	0.012		E_{lim1}	100.2±1.1988
α_S	0.9355±0.2671	1.25		α_1	0.7505±0.0205
$Y_{E/S}$	2.17	2.17		K_{st}	0.0007528±0.0000
$Y_{CO2/S}$	2.17	2.17		k_N	0.03373±0.0004
k_3	0.001033±0.0001	0.001		K_{N1}	0.008628±0.0003
K_N	0.04105±0.0147	0.03		g_1	0.2018±0.0034
K_{NI}	0.02635±0.0096	0.035		g_2	0.1243±0.0027
α_N	1.195±0.295	1.5		g_3	$6g_1$-1
Q_0	0.0001347±0.000	-		k_{NST}	(7.661±0.0046).E-5
E_{max}	94.67±8.1371	-		E_{lim2}	90.55±2.6018
k_{nst}	1.0024±0.5750	-		α_2	(4.71±2.857)
k_{dnst}	1.987±0.5	-		N_{intra1}	0.0005719±0.0000
K_{nst}	10.76±7.1421	-		κ	0.35002±0.0015
Y_{nst}	694.8±118.2153	-		g_S	1.909±0.0035
Q_{0nst}	0.0001334±0.000	-			
α_1	0.0004795±0.0001	-			
κ	0.03±0.0057	-			

4.2 Models comparison

The Figure shows the comparison of the two calibrated models' simulation with experimental data and simulation of Brou's and Malherbe's models. The Brou's and Malherbe's models were calibrated with the same data as models 1 and 2 to better compare them. Graphically, models 1 and 2 fit experimental data well and simulate slightly better experimental data than Brou's and Malherbe's models. Models 1 and 2 fit less on MS70+100 than for other fermentations on CO_2 production Brou's model underestimates the final CO_2 production for the four fermentations. The Malherbe's model simulates well the CO_2 production for two fermentations (MS70+100 and MS140+50). However, Malherbe's model simulates badly the CO_2 production after nitrogen addition for the other two fermentations (MS140+150 and MS210+100), where added nitrogen is important. Models 1 and 2 fit the CO_2 production rate better before nitrogen addition than after nitrogen addition especially for maximum value of CO_2 production rate. Model 1 seems to be slightly better than model 2 for CO_2 production and for CO_2 production rate. Model 1 and 2 simulate better CO_2 production rate than Malherbe's and Brou's models after nitrogen addition. Model 1 and 2 simulate well nitrogen consumption during the first part of fermentation unlike the Malherbe's model but, after nitrogen addition, the simulated consumption is faster than experimental data. Brou's model simulates also the nitrogen consumption too quickly after the nitrogen addition.

Models 1 and 2 seem to have equivalent performance on nitrogen. Concerning simulation of biomass dynamics, Model 1 underestimates the biomass production rate while model 2 tends to overestimate this rate after nitrogen addition. Both models overestimate maximum biomass produced for MS210+100 and for MS70+100 but estimate well maximum biomass produced

for MS140+50 and MS140+150. Brou's model overestimates the biomass whereas Malherbe's model underestimates the biomass production.

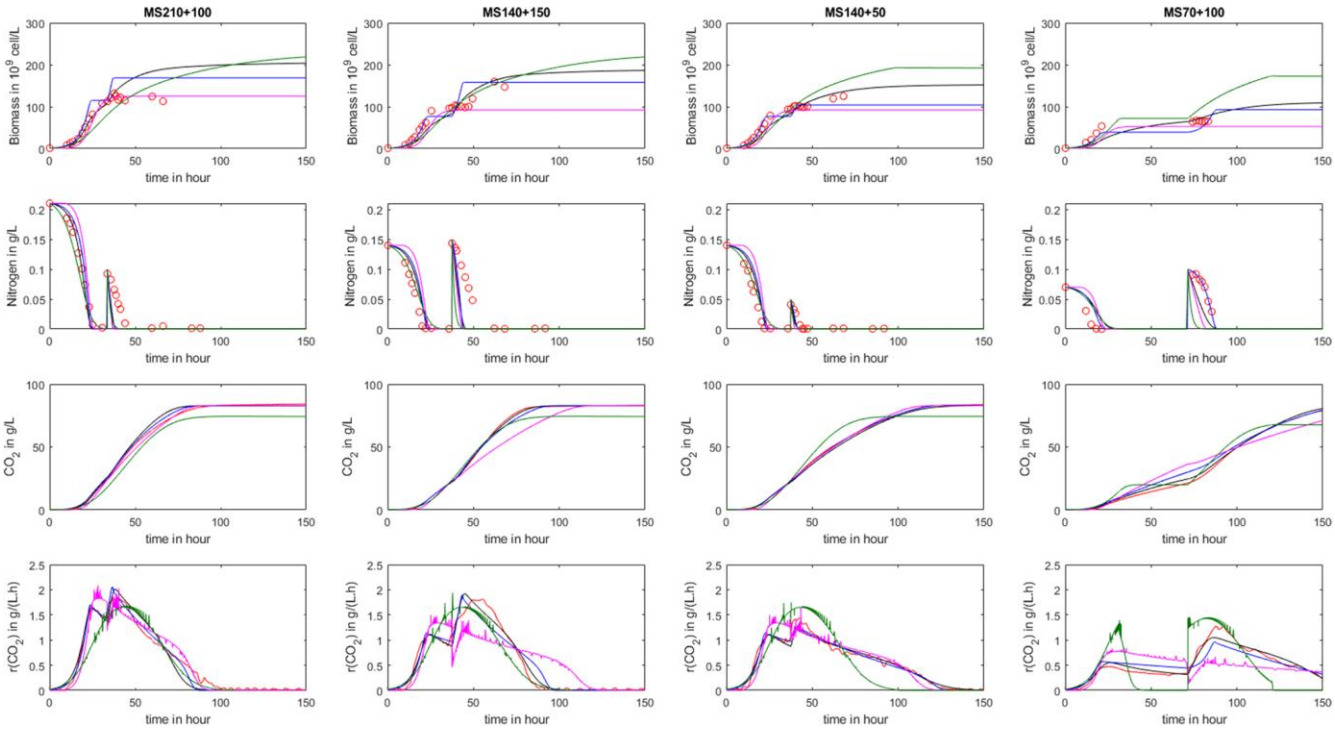

Figure 2 Dynamic evolution of biomass, nitrogen, CO_2 and CO_2 production rate for the experimental data (in red) and data simulated by models 1 (in black), model 2 (in blue), Malherbe's model (in pink) and Brou's model (in green) for the four calibration fermentatations

The Table 4 shows the NRMSE values for each variable for all fermentations for Models 1 and 2.
Model 1 is slightly better than model 2 for the whole fermentations and for the whole variables (NRMSE values of 0.208 for model 1 and 0.241 for model 2 cf. Table 4) and for each variable. Model 1 better describes kinetic evolution of experimental data than model 2 for nitrogen for the four fermentation and for biomass for three fermentations (not for MS140+50). Regarding the NRMSE for CO_2, r_{CO2} and for the whole variables for MS140+150 and for MS70+100, Model 1 is closer to the data than the model 2 while model 2 is slightly better for MS210+100 and for MS140+50.

Table 4 NRMSE values applied to each variable for each fermentation, to all of the variables for each fermentation, to each variable for all of the fermentations and to all of the variables for all of the fermentations and for Models 1 and 2.

Model	Fermentations	CO_2 in g/L	r_{CO2} in g/(L.h)	Biomass in 10^9 cell/L	Nitrogen in g/L	All variables
Model 1	MS210+100	0.0320	0.0773	0.199	0.112	0.121
	MS140+150	0.00838	0.0587	0.109	0.251	0.123
	MS140+50	0.0158	0.0548	0.0867	0.0990	0.0901
	MS70+100	0.0222	0.0826	0.208	0.213	0.120
	All fermentations	0.0391	0.137	0.292	0.331	0.208
Model 2	MS210+100	0.0192	0.0494	0.245	0.126	0.101
	MS140+150	0.0204	0.0902	0.156	0.254	0.138
	MS140+50	0. 00556	0.0459	0.0773	0.117	0.0836
	MS70+100	0.0757	0.195	0.103	0.155	0.219
	All fermentations	0.0464	0.161	0.357	0.349	0.241

According to the AIC values (Table 5), Models 1 and 2 are better than Malherbe's and Brou's Models with higher nitrogen addition: for instance, for MS+150 the AIC value for Brou's Model and Malherbe's Model are approximately two times the value for Model 1 and for Model 2. Models 1 and 2 are slightly better than Brou's and Malherbe's Models for fermentations with a lower nitrogen addition. Models 1 and 2, Malherbe's and Brou's Models are equivalents for the biomass and for nitrogen. Models 1 and 2 present better performance than Brou's and Malherbe's Model for CO_2 production rate.

Table 5 AIC values applied to each variable for each fermentation and for Models 1, 2, of Brou and of Malherbe.

Model	Fermentations	CO_2 in g/L	r_{CO2} in g/(L.h)	Biomass in 10^9 cell/L	Nitrogen in g/L
Model 1	MS210+100	2234	-437	254	272
	MS140+150	1012	-718	242	274
	MS140+50	1597	-1000	233	273
	MS70+100	1948	-1020	104	184
Model 2	MS210+100	1761	-834	262	274
	MS140+150	1839	-301	250	276
	MS140+50	637	-1159	233	275
	MS70+100	2653	-409	136	186
Brou's Model	MS210+100	3147	-142	237	268
	MS140+150	3027	-59	241	270
	MS140+50	3082	245	240	269
	MS70+100	3037	433	124	180
Malherbe's Model	MS210+100	1736	-416	240	272
	MS140+150	3230	446	220	274
	MS140+50	1486	-462	220	27
	MS70+100	3250	295	129	184

5 Model sensitivity

Sensitivity analysis was done to find parameters that play the main role in the models. Moreover, as the model will be used for online optimization, the model sensitivity to parameter variations must be studied. In this work the sensitivity coefficients are calculated for biomass, nitrogen, CO_2 and r_{CO2} and for a modification of each parameter value between -10% and 10% with a step of 0.5%. For the model 1, the sensitivity analysis was not applied to parameter k_1 because this parameter depends

on temperature and we use only one temperature. For the model 2, the sensitivity analysis was not applied to the parameter g_3 because this parameter is calculated from the parameter g_1 ($g_3 = 6g_1 - 1$).
Figure 3 and **Erreur ! Source du renvoi introuvable.** represent the sensitivity coefficients calculated for CO_2 release and for each parameters of model 1 and 2 respectively. Model 1 is sensitive to variations of four parameters (k_2, N_{intra0}, Q_{0nst}, Y_{nst}). Model 2 is sensitive to variations of three parameters (k_2, k_3 and g_2) and diverges for some modifications of two parameters (E_{lim2} and g_S). Model 1 seems to be less sensitive than model 2 because model 2 diverges for some modifications of two parameters values (E_{lim2} and g_S).

For the model 1, nitrogen is the most sensitive variable to changes in parameters as for all parameters the sensitivity coefficient takes an absolute value of more than 1 for at least one variation of the parameter. Increasing Q_0 or decreasing Q_{0nst} result in the greatest variation of the sensitivity coefficient for nitrogen.
Biomass is not very sensitive to the variation of the parameters: only two parameters modifications leads to a sensitivity coefficient of more than 1 in absolute value: Q_0 and Q_{0nst}.
The CO_2 production rate r_{CO2} is not sensitive to the modification of the value of most of the parameters except for the diminution of k_2, Q_0 and Y_{nst} that can lead respectively to a sensitivity coefficient of -8.88, -4.21 and -2.90 for a diminution of 10% of the three parameters values.
CO_2 is the variable least sensitive to variations in parameters values: for only five parameters, the value modification can lead to a sensitivity coefficient more than 0.1 in absolute value but none of them induce a sensitivity coefficient more than 1 in absolute value.
For the model 2, increasing g_S by more than 7% or decreasing E_{lim2} by more than 6.5% lead to the divergence of model 2. As for model 1, nitrogen is very sensitive to parameters value modification: most of the modification lead to a sensitivity coefficient more than 1 in absolute value. Nitrogen is most sensitive to modification of g_2: sensitivity coefficient can be more than 100.
Biomass is not very sensitive to parameters value modification: no modification leads to a sensitivity coefficient of more than 1 in absolute value.
Concerning r_{CO2}, the modifications of 6 parameters lead to a sensitivity coefficient more than 1 in absolute value especially the modification of k_2, E_{lim2} and g_2 that lead to a sensitivity coefficient more than 20 in absolute value.
CO_2 is not sensitive to the modification of any parameters: no parameters value modification lead to a sensitivity coefficient more than 0.5 in absolute value.

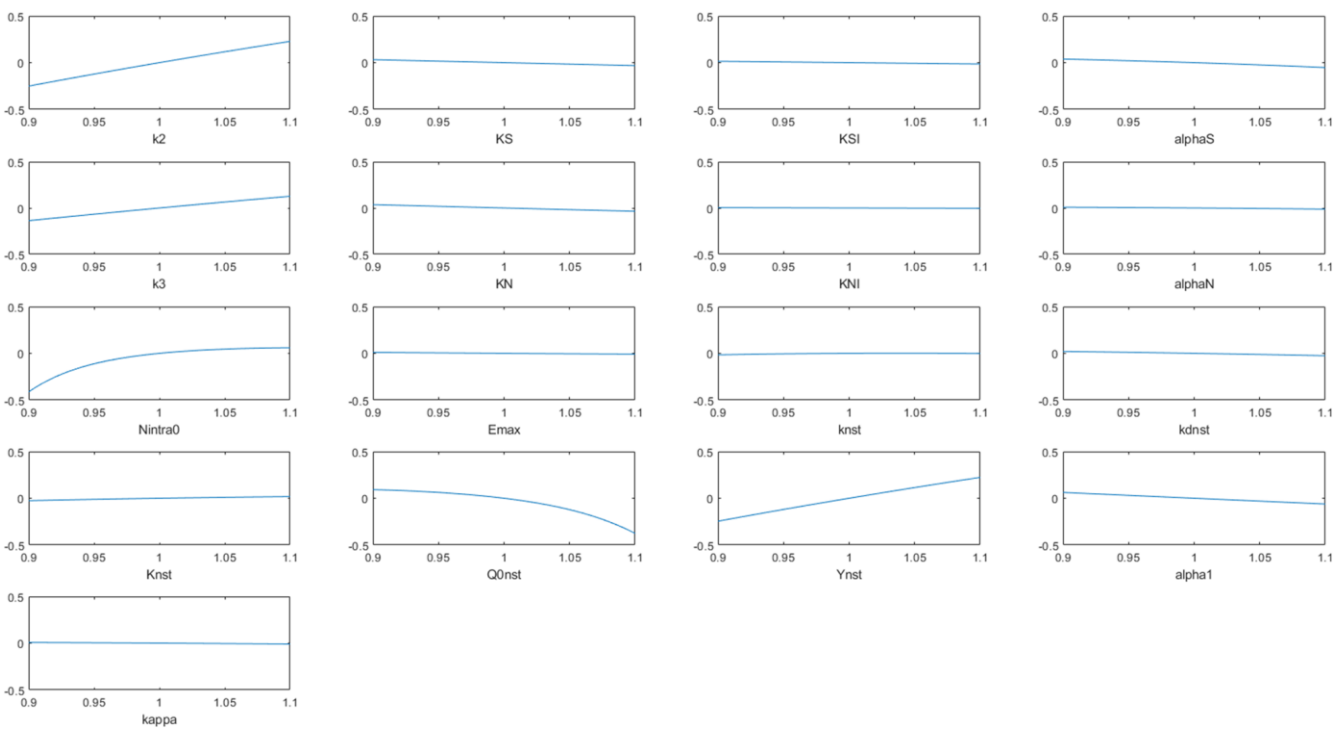

Figure 3 Sensitivity coefficients for each model 1 parameters and for CO_2 release

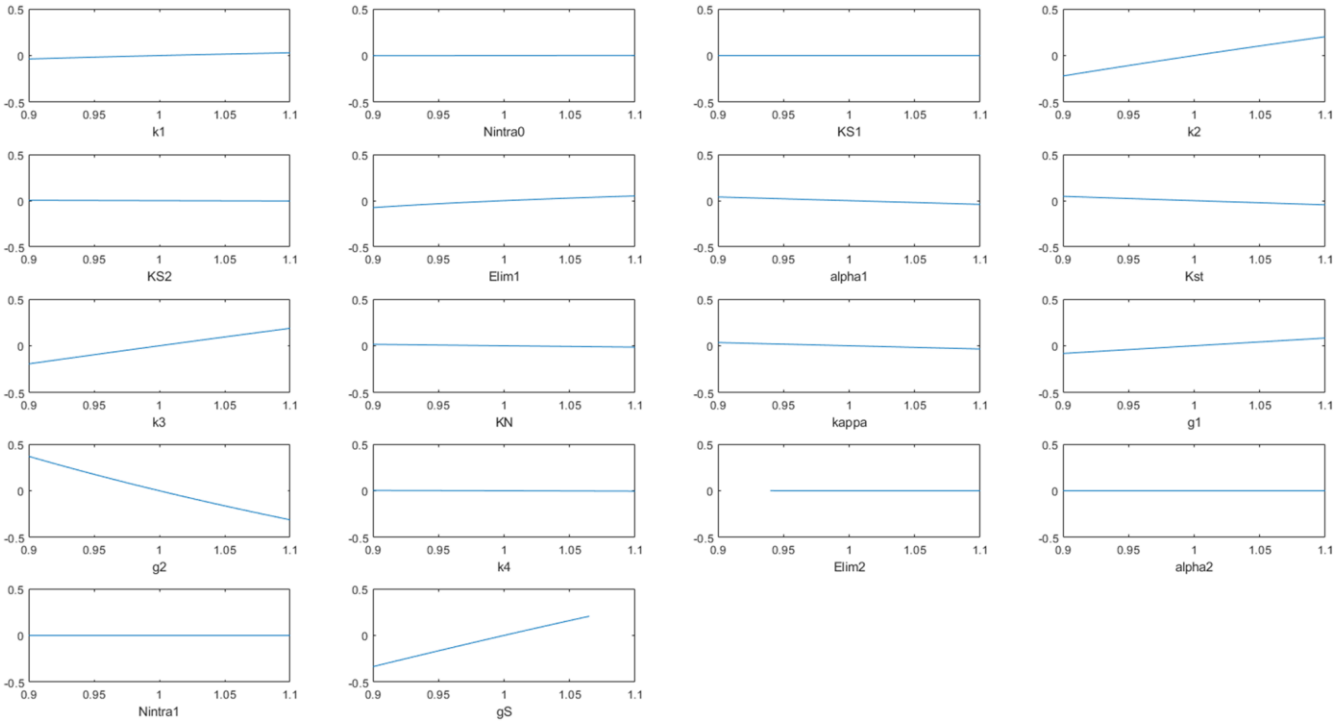

Figure 4 Sensitivity coefficients for each model 2 parameters and for CO_2 release

6 Models validation

Models 1 and 2 are validated and compared with Malherbe's [8] and Brou's [11] models recalibrated with the same calibration data as models 1 and 2 on data from 5 fermentations with nitrogen addition. The fermentations are made with three initial nitrogen concentrations (70, 140 and 210 mg/L), three added nitrogen concentrations (50, 100 and 150 mg/L) and two timing of nitrogen addition (20 and 35 g/L of CO_2).

Erreur ! Source du renvoi introuvable. shows data generated by the four models and experimental data for the five calibration fermentations. Graphically Malherbe's model simulates well the maximal biomass concentration and Brou's model overestimate maximal biomass concentration before nitrogen addition. Malherbe's model does not predict biomass regrowth because its biomass equation does not take into account a nitrogen add. Brou's model simulates a biomass regrowth after nitrogen addition but overestimates strongly the biomass production. Model 2 predicted well the maximal biomass after the first part of the biomass growth whereas model 1 simulates too slowly biomass production in the first part of biomass growth. After nitrogen addition model 2 is closer to the experimental biomass measure than the model 1.

Malherbe's model estimates well CO_2 production for three fermentations (MS140+50 and MS210+100 with a nitrogen addition at 20 g/L of CO_2 released and MS140+100 with a nitrogen addition at 35 g/L CO_2 released) but estimates poorly the CO_2 production for MS70+100 with a nitrogen addition at 20 g/L of CO_2 released during the whole fermentation and MS140+150 with a nitrogen addition at 20 g/L of CO_2 released after nitrogen addition. Brou's model estimates well CO_2 production before nitrogen addition but underestimates CO_2 production after nitrogen addition and the final CO_2 production. Models 1 and 2 simulate well CO_2 production all around the fermentation but they slightly overproduce CO_2 after nitrogen addition.

Malherbe's model does not predict very well the CO_2 production rate before the nitrogen addition. Malherbe's model overestimates the V_{max}. Brou's model simulates poorly the CO_2 production rate for the five fermentations. The nitrogen addition has no effect on CO_2 production rate simulated by Brou's and Malherbe's models. Models 1 and 2 simulate well CO_2 production rate during the whole fermentation. Models 1 and 2 predict well the CO_2 production rate during the whole fermentations. Models 1 and 2 predict well the V_{max} value during the first phase of growth and the time at which V_{max} are reached before and after nitrogen addition but during the second growth phase after nitrogen addition, V_{max} is less well estimated than before nitrogen add by models 1 and 2.

Globally models 1 and 2 simulate the five validation fermentations of calibration better than Malherbe's and Brou's models. Models 1 and 2 simulate very well the fermentation with a nitrogen add at 35 g/L of CO_2 released and can be extended to fermentations with different nitrogen adds timings.

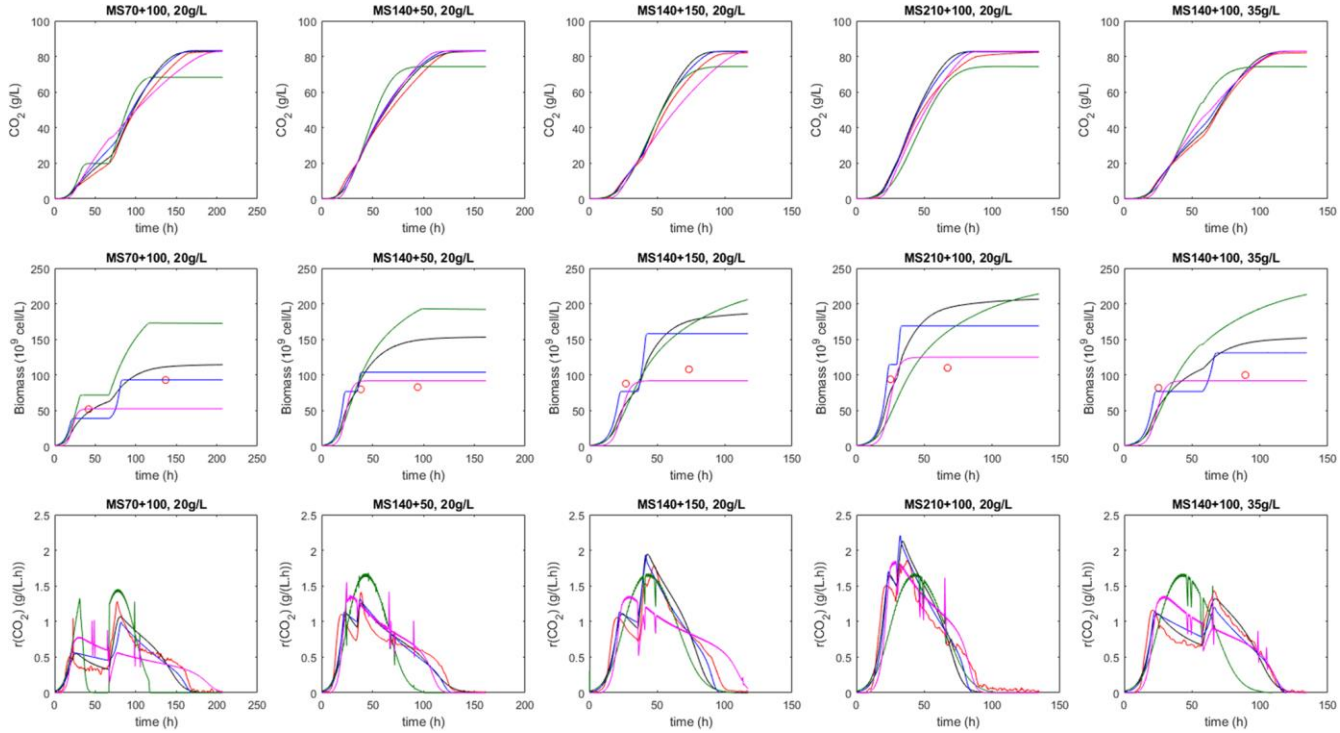

Figure 5 Dynamic evolution for CO_2 production, biomass production and CO_2 production rate for the experimental data (in red) and data simulated by models 1 (in black), 2 (in blue), Malherbe's model (in green) and Brou's model (in magenta). The first line represents the CO_2 production, the second line the biomass production and the third line the CO_2 production rate.

The NRMSE values for CO_2, CO_2 production rate and biomass for each fermentation are presented in Table 6. Models 1 and 2 simulate well CO_2 production (NRMSE values go respectively from 0.0186 to 0.0474 and from 0.0287 to 0.0465). They fit better to experimental data than Malherbe's and Brou's models (NRMSE values from 0.0299 to 0.637 and from 0.0519 to 115 for Malherbe's and Brou's Models respectively). Models 1 and 2 fit better to experimental data than Malherbe's and Brou's models for models for the CO_2 production rate with NRMSE for models 1 and 2 (NRMSE values go respectively from 0.0399 to 0.110 and from 0.0398 to 0.109) lower than those obtained for Malherbe's and Brou's Model (NRMSE values from 0.116 to 0.208 and from 0.143 to 0.335 for Malherbe's model and Brou's Models respectively).

The biomass is badly simulated by models 1 and 2 (NRMSE values go respectively from 0.119 to 0.479 and from 0.0707 to 0.362), as Malherbe's and Brou's Models. The nitrogen addition at 35g/L of CO_2 release does not change the models 1 and 2 performances: the NRMSE values are not upper than the NRMSE values for a nitrogen add at 20g/L of CO_2 release. Models 1 and 2 are validated on CO_2, r_{CO2} and biomass (two measures) data from 5 fermentations with nitrogen addition for two different fermentation progress. They allow to simulate a nitrogen addition at 35g/L of CO_2 released despite being calibrated on fermentation with nitrogen addition at 20g/L of released CO2. Models 1 and 2 present a better adequation to experimental data than Brou's and Malherbe's model. Model 1 are slightly better than model 2 for CO_2 production (NRMSE average value respectively of 0.0365 and 0.0404) whereas model 2 are slightly better for r_{CO2} and biomass than model 1 (NRMSE average value respectively of 0.0804 and 0.0756 for CO_2 production rate and of 0.235 and 0.371 for biomass).

Table 6 NRMSE calculated for CO_2, CO_2 production rate and biomass for models 1 and 2, Brou and Malherbe

Fermentation			NRMSE											
N_0	CO_{2aj}	N_{aj}	CO_2				r_{CO2}				Biomass			
mg/L	g/L	mg/L	Model 1	Model 2	Brou	Malherbe	Model 1	Model 2	Brou	Malherbe	Model 1	Model 2	Brou	Malherbe
70	20	100	0.0372	0.0465	0.107	0.066	0.0399	0.0398	0.267	0.208	0.119	0.0707	0.626	0.306
140	20	50	0.0271	0.0393	0.099	0.0474	0.0961	0.109	0.280	0.164	0.479	0.267	0.926	0.121
140	20	150	0.0474	0.0352	0.0519	0.637	0.106	0.0859	0.166	0.181	0.443	0.293	0.463	0.172
140	35	100	0.0186	0.0287	0.115	0.0502	0.0499	0.0499	0.354	0.202	0.358	0.181	0.648	0.193
210	20	100	0.0522	0.0381	0.0742	0.0300	0.110	0.0936	0.143	0.116	0.458	0.362	0.455	0.202

According to AIC values, Models 1 and 2 predict better than Brou's and Malherbe's Models the CO_2 production rate: the AIC values goes from -766 to -90.1 for Model 1, from -630 to -215 for Model 2, from 123 to 638 for Brou's Model and from -44.0 to 239 for Malherbe's Model.

Models 1 and 2 are better than Brou's Model for all fermentations and than Malherbe's Model to predict CO_2 for almost all fermentations: Malherbe's Model is better than Models 1 and 2 for MS210+100 with a nitrogen addition at 20 g/L of CO_2 released.

Table 7 AIC values calculated for CO_2, CO_2 production rate and biomass for models 1 and 2, of Brou and of Malherbe

Fermentation			AIC											
N_0	CO_{2aj}	N_{aj}	CO_2				r_{CO2}				Biomass			
mg/L	g/L	mg/L	Model 1	Model 2	Brou	Malherbe	Model 1	Model 2	Brou	Malherbe	Model 1	Model 2	Brou	Malherbe
70	20	100	3180	3458	4481	3888	-766	-630	466	153	52,0	52,5	57,9	55,1
140	20	50	2200	2565	3468	2663	-528	-404	511	-8,71	57,1	56,1	59,0	50,9
140	20	150	1991	1785	2056	2196	-134	-282	181	239	57,5	58,0	57,3	53,4
140	35	100	1509	1860	2967	2301	-609	-385	638	183	56,4	56,1	58,4	53,5
210	20	100	2339	2087	2619	1891	-90,1	-215	123	-44,0	58,1	58,8	57,3	54,1

7 Conclusions

Two models of fermentation in oenological conditions were proposed in this work. They are based on a storage compartment of nitrogen in yeast cells and simulate the macroscopic temporal evolution of biomass, sugar, ethanol, carbon dioxide, nitrogen, intra-cellular nitrogen and cell activity by differential equations. They consider effect of nitrogen addition during fermentation on growth and production kinetics of CO_2 and ethanol. The two models are robust for most parameters and have been validated on experimental data with good fitting on CO_2 release and production rate.

Even though both developed models have good performance on the validation fermentations, Model 1 is slightly better than Model 2 on CO_2 and CO_2 production rate (NRMSE average value respectively of 0.0365 and 0.0404) whereas model 2 are slightly better for CO_2 production rate and biomass than model 1 (NRMSE average value respectively of 0.0804 and 0.0756 for CO_2 production rate and of 0.235 and 0.371 for biomass. The Models 1 and 2 present better performance than Malherbe's and Brou's Models according to AIC values even if they have been calibrated with the same data as models 1 and 2. In addition, Model 1 is less sensitive than Model 2 to changes in parameters.

Funding

This research project was funded by the French National Research Agency (ANR) under grant agreement STARWINE (ANR-18-CE10-0013).

Data availability

The datasets presented in this study can be found in online repositories. The names of the repository/repertories and accession number(s) can be found below: https:// www. ncbi. nlm. nih.gov/ geo., GSE189010.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Monteiro, F.F. and L.F. Bisson, *Amino Acid Utilization and Urea Formation During Vinification Fermentations.* American Journal of Enology and Viticulture, 1991. **42**(3): p. 199-208.
2. Agenbach, W.A., *A study of must nitrogen content in relation to incomplete fermentations, yeast production and fermentation activity*, 1977, South African Society for Enology and Viticulture: Stellenbosch (ZA).
3. Bely, M., J.M. Sablayrolles, and P. Barre, *Automatic Detection of Assimilable Nitrogen Deficiencies during Alcoholic Fermentation in Enological Conditions.* Journal of Fermentation and Bioengineering, 1990. **70**(4): p. 246-252.
4. Mendes-Ferreira, A., A. Mendes-Faia, and C. Leão, *Growth and fermentation patterns of Saccharomyces cerevisiae under different ammonium concentrations and its implications in winemaking industry.* Journal of Applied Microbiology, 2004. **97**(3): p. 540-545.
5. Bisson, L.F., *Stuck and sluggish fermentations.* Américan Journal of enology and viticulture, 1999(50): p. 107-119.
6. Crépin, L., T. Nidelet, I. Sanchez, S. Dequin, and C. Camarasa, *Sequential use of nitrogen compounds by Saccharomyces cerevisiae during wine fermentation : a model based on kinetic and regulation characteristics of nitrogen permeases.* Applied and Environmental Microbiology, 2012. **77**: p. 8102-8111.
7. Carrau, F.M., K. Medina, L. Farina, E. Boido, P.A. Henschke, and E. Dellacassa, *Production of fermentation aroma compounds by Saccharomyces cerevisiae wine yeasts: effects of yeast assimilable nitrogen on two model strains.* FEMS Yeast Research, 2008. **8**(7): p. 1196-207.
8. Malherbe, S., V. Fromion, N. Hilgert, and J.M. Sablayrolles, *Modeling the effects of assimilable nitrogen and temperature on fermentation kinetics in enological conditions.* Biotechnology and Bioengineering, 2004. **86**(3): p. 261-72.
9. Commission, E., *Commission Delegated Regulation (EU) 2019/934 of 12 March 2019 supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as regards wine-growing areas where the alcoholic strength may be increased, authorized oenological practices and restrictions applicable to the production and conservation of grapevine products, the minimum percentage of alcohol for by-products and their disposal, and publication of OIV files*, 2019.
10. David, R., D. Dochain, J.R. Mouret, A. Vande Wouwer, and J.M. Sablayrolles, *Nitrogen-backboned modeling of wine-making in standard and nitrogen-added fermentations.* Bioprocess and biosystems engineering, 2014. **37**(1): p. 5-16.
11. Brou, P.R.J., P. Taillandier, S. Beaufort, and C. Brandam, *Modelling of S. cerevisiae and T. delbrueckii pure culture fermentation in synthetic media using a compartmental nitrogen model.* OENO One, 2020. **54**: p. 299–311.
12. Bisson, L.F. *Influence of nitrogen on yeast and fermentation of grapes*. in *Proceedings of the International Symposium on Nitrogen in Grapes and Wine: Seattle, Washington, Usa 18-19 june 1991*. 1991. American Society for Enology and Viticulture, ASEV.
13. Leão, C. and N. Van Uden, *Effects of ethanol and other alkanols on the ammonium transport system of Saccharomyces cerevisiae.* Biotechnology and Bioengineering, 1983. **25**(8): p. 2085-2089.
14. Bisson, L.F., *Glucose transport in Saccharomyces cerevisiae and the role of potassium in stuck fermentation.* Proceedings of the 2000 Entretiens Scientifiques Lallemand, Krems, Austria, 2000: p. 27-33.

15. Ansanay-Galeote, V., B. Blondin, S. Dequin, and J.-M. Sablayrolles, *Stress effect of ethanol on fermentation kinetics by stationary-phase cells of Saccharomyces cerevisiae.* Biotechnology Letters, 2001. **23**(9): p. 677-681.
16. Aiba, S., M. Shoda, and M. Nagatani, *Kinetics of product inhibition in alcohol fermentation.* Biotechnology and Bioengineering, 1968. **10**(6): p. 845-864.
17. Boulton, R., *The Prediction of Fermentation Behavior by a Kinetic Model.* American Journal of Enology and Viticulture, 1980. **31**(1): p. 40-45.
18. Caro, I., L. Pérez, and D. Cantero, *Development of a kinetic model for the alcoholic fermentation of must.* Biotechnology and Bioengineering, 1991. **38**(7): p. 742-748.
19. Colombié, S., S. Malherbe, and J.-M. Sablayrolles, *Modeling Alcoholic Fermentation in Enological Conditions: Feasibility and Interest.* American Journal of Enology and Viticulture, 2005. **56**(3): p. 238-245.
20. Marín, M.R., *Alcoholic Fermentation Modelling: Current State and Perspectives.* American Journal of Enology and Viticulture, 1999. **50**(2): p. 166-178.
21. Sevely, Y., J.B. Pourciel, G. Rauzy, and J.P. Bovee, *Modelling, Identification and Control of the Alcohol Fermentation in a Cascade Reactor.* IFAC Proceedings Volumes, 1981. **14**(2): p. 2821-2828.
22. Williams, D., P. Yousefpour, and E.M.H. Wellington, *On-line adaptive control of a fed-batch fermentation of Saccharomyces cerevisiae.* Biotechnology and Bioengineering, 1986. **28**(5): p. 631-645.
23. Cramer, A.C., S. Vlassides, and D.E. Block, *Kinetic model for nitrogen-limited wine fermentations.* Biotechnology and bioengineering, 2002. **77**(1): p. 49-60.
24. Bely, M., J.M. Sablayrolles, and P. Barre, *Description of alcoholic fermentation kinetics-its variability ad significance.* American Journal ofr Enology and Viticulture, 1990. **70**: p. 246-252.
25. Mouret, J.R., C. Camarasa, M. Angenieux, E. Aguera, M. Perez, V. Farines, and J.M. Sablayrolles, *Kinetic analysis and gas–liquid balances of the production of fermentative aromas during winemaking fermentations: Effect of assimilable nitrogen and temperature.* Food Research International, 2014. **62**: p. 1-10.
26. Sablayrolles, J.M., P. Barre, and G. P., *Design of a laboratory automatic system for studying alcoholic fermentations in anisothermal enological conditions.* Biotechnology Techniques, 1987. **1**: p. 181-184.
27. Godillot, J., I. Sanchez, M. Perez, C. Picou, V. Galeote, J.-M. Sablayrolles, V. Farines, and J.R. Mouret, *The Timing of Nitrogen Addition Impacts Yeast Genes Expression and the Production of Aroma Coumpounds During Wine Fermentation.* Frontiers in Microbiology, 2022. **13**.
28. Shampine, L.F. and M.W. Reichelt, *The MATLAB ODE Suite.* SIAM Journal on Scientific Computing, 1997. **18**(1): p. 1-22.
29. Armstrong, J.S. and F. Collopy, *Error measures for generalizing about forecasting methods: Empirical comparisons.* International Journal of Forecasting, 1992. **8**(1): p. 69-80.
30. Akaike, H., *A New Look at the Statistical Model Identification.* IEEE Tnrasactions on Automatic Control, 1974. **19**(6): p. 716-723.
31. Bernard, O., Z. Hadj-Sadok, D. Dochain, A. Genovesi, and J.P. Steyer, *Dynamical model development and parameter identification for an anaerobic wastewater treatment process.* Biotechnology and bioengineering, 2001. **75(4)**: p. 424-438.