Modelling of *S. cerevisiae* and *T. delbrueckii* pure culture fermentation in synthetic media using a compartmental nitrogen model

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**Aim:** The objective of the present work is to propose a model describing the evolution of the pure culture fermentation of two oenological yeasts: *S. cerevisiae* and *T. delbrueckii*.

**Methods and results:** For both yeasts, pure culture fermentation was performed in a synthetic medium with different initial concentrations of yeast available nitrogen. The datasets obtained from those experiments were used to identify the parameters of the proposed model.

**Conclusions:** The developed comprehensive model of wine-making fermentation is based on the partition of assimilated nitrogen between the constitutive and the storage compartments. It efficiently describes the evolution of *S. cerevisiae* and *T. delbrueckii* pure cultures. This mass-balance model provides a stoichiometric approach in biomass production, unlike nitrogen backbone models used in winemaking. Moreover, it gives an estimation of non-accessed data such as nitrogen partition between vacuole and cytosol during *T. delbrueckii* fermentation.

**Significance and impact of the study:** The developed model is robust enough to precisely describe the fermentation evolution of two pure culture yeasts and therefore has future potential for modelling mixed culture fermentations of *S. cerevisiae* and *T. delbrueckii*.

**ABSTRACT**

**KEYWORDS**

modelling, fermentation, *S. cerevisiae, T. delbrueckii*

**NOMENCLATURE**

- \( a \): correlation coefficient linking living cells concentration to mass concentration (10^9 cells/g)
- \( E \): ethanol concentration (g/L)
- \( G \): glycerol concentration (g/L)
- \( K_{g} \): constant of glycerol production kinetics (1/h)
- \( K_{n} \): constant of nitrogen assimilation kinetics (L/mol/h)
- \( K_{f} \): constant of fermentation kinetics (1/h)
- \( K_{\mu} \): constant of growth kinetics (1/h)
- \( K_{E} \): constant of growth inhibition by ethanol (g/L)
- \( K_{EG} \): constant of glycerol production inhibition (g/L)
- \( K_{Es} \): constant of ethanol production inhibition by ethanol (g/L)
- \( K_{NS} \): constant of ethanol production limitation by stored nitrogen (mg N/10^9 cells)
- \( K_{S} \): constant of ethanol production by sugar (g/L)
- \( K_{SG} \): constant of glycerol production limitation by sugar (g/L)
- \( K_{G} \): constant of growth limitation by stored nitrogen (mg N/10^9 cells)
- \( M \): yeast molar mass (g/mol)
- \( M' \): yeast constitutive molar mass (g/mol)
- \( N \): nitrogen concentration in the medium (mg N/L)
- \( N_{S} \): stored nitrogen concentration (mg N/L)
- \( S \): sugar concentration (g/L)
- \( X \): yeast living cells concentration (10^9 cells/L)
- \( X_{\text{max}} \): maximum yeast living cells concentration (10^9 cells/L)
- \( X_{n} \): yeast constitutive molar concentration (mol/L)
INTRODUCTION

Wine fermentation is a widely studied process in which hexoses are mainly converted into ethanol and carbon dioxide. Saccharomyces species are the yeasts most commonly used in winemaking, since they are tolerant to high ethanol levels (Pretorius, 2000). T. delbrueckii is of increasing interest, because it has been shown to have a positive impact on the organoleptic quality of wines and to produce low levels of undesired compounds, such as acetic acid, ethyl acetate, acetaldehyde, acetoin, hydrogen sulphide and volatile phenols that lessen off-flavours (Ciani and Picciotti, 1995; Renault et al., 2009; Ciani and Maccarelli, 1997; Loira et al., 2015; Canonico et al., 2016; Dutraive et al., 2019; Benito et al., 2019).

Yeasts are living organisms whose activity requires nutrients to provide both the basic elements and energy required for various biochemical syntheses. Nitrogen is one such nutrient, being essential for metabolism and growth during alcoholic fermentation (Jiranek et al., 1995). A lot of studies have been undertaken, especially with S. cerevisiae, to help understand, quantify and overcome nitrogen limitation during fermentation (Anderson and Kirsop, 1974; Henschke and Jiranek, 1993; Albers et al., 1996; Arias-Gil et al., 2007; Taillardier et al., 2007; Carrau et al., 2008; Bergdahl et al., 2012; Crepin et al., 2012; Casalta et al., 2013; Su et al., 2020). A lack of nitrogen sources is the main cause of sluggish fermentation (Mendes-Ferreira et al., 2004; Jolly et al., 2014). Yeasts completely consume nitrogen during the first two days of fermentation (Monteiro and Bisson, 1991), and the assimilated nitrogen is distributed among cytosol, membranes and vacuoles. During fermentation there is an intracellular amino acids concentration gradient between the cytosol and the more concentrated vacuoles (Wiemken and Dürr, 1974). This compartmentalisation contributes to the regulation of the activity of many enzymes involved in the metabolism (Sumrada and Cooper, 1982).

Several mathematical models have been proposed in order to understand, simulate and control the fermentation process in winemaking (Sevely et al., 1981; Williams et al., 1986; Caro et al., 1991; Marin, 1999; Colombié et al., 2005; Coleman et al., 2007; Goelzer et al., 2009). They describe the macroscopic evolution of biomass, sugar, ethanol, carbon dioxide and sometimes nitrogen and glycerol. Among these, attention has focused on models in which growth is based on nitrogen sources (Cramer et al., 2002; Malherbe et al., 2004; David et al., 2013; Mouret et al., 2015; Henrques et al., 2018). They describe the evolution of substrates and metabolites using biochemical reactions and mass balances. Even under limiting nitrogen conditions, nitrogen backbone models do not include mass balance and biochemical reaction to describe biomass evolution. The relationship between growth and nitrogen concentration is made by empirically linking the initial nitrogen concentration to the maximum living cell or to the maximum growth rate.

This paper describes a comprehensive kinetic model in which yeast growth is based on assimilated nitrogen partition. It aims to predict the evolution of major components and to contribute to understanding nitrogen limitation during winemaking fermentation. Experimental data has been acquired and a model has been developed for pure cultures of S. cerevisiae and T. delbrueckii.

MATERIALS AND METHODS

1. Yeast

Two commercial oenological yeasts were used in this study: Torulaspora delbrueckii Zymaflore alpha® supplied by Laffort S.A.S., France, and Saccharomyces cerevisiae QA23® supplied by Lallemand S.A.S., France. The yeasts were maintained on sterile YPD agar slants [yeast extract 1 % (w/v), peptone 1 % (w/v), glucose 2 % (w/v) and agar 2 % (w/v)] at 4 °C. When the inoculation cultures had been prepared, each yeast was transferred from the agar slant to a flask containing 50 mL of YPD [yeast extract 1 % (w/v), peptone 1 % (w/v), glucose 2 % (w/v)]. The yeasts were incubated at 25 °C with agitation (130 rpm) for 11 h. Subsequently, they were transferred to an Erlenmeyer flask containing 300 mL of YPD to give an initial concentration of 5.10^6 cells/mL, and then incubated with agitation (130 rpm) at 25 °C for 16 h.

2. Medium

Two synthetic media, MS170 and MS300, were used. They contained (per litre): 110 g glucose, 110 g fructose, 6 g L-malic acid, 6 g citric acid, mineral salts (750 mg KH₂PO₄, 500 mg K₂SO₄, 250 mg MgSO₄·7H₂O, 155 mg CaCl₂·2H₂O,
200 mg NaCl, 4 mg MnSO₄·H₂O, 4 mg ZnSO₄·7H₂O, 1 mg CuSO₄·5H₂O, 1 mg KI, 0.4 mg CoCl₂·6H₂O, 1 mg H₃BO₃, and 1 mg (NH₄)₆Mo₇O₂₄), vitamins (20 mg myo-inositol, 2 mg nicotinic acid, 1.5 mg calcium pantothenate, 0.25 mg thiamin-HCl, 0.25 mg pyridoxine–HCl, and 0.003 mg biotin), anaerobic growth factors (15 mg ergosterol, 443 μg oleic acid, and 1 mL of a Tween80/ethanol solution (1:1, v/v)). The assimilable nitrogen was in the form of ammonium ions, NH₄Cl, and a concentrated solution of 19 α- amino acids. The composition of the concentrated amino acid solution was (per litre of solution): 1.4 g tyrosine, 13.7 g tryptophan, 2.5 g isoleucine, 3.4 g aspartic acid, 9.2 g glutamic acid, 28.6 g arginine, 3.7 g leucine, 5.8 g threonine, 1.4 g glycine, 38.6 g glutamine, 11.1 g alanine, 3.4 g valine, 2.4 g methionine, 2.9 g phenylalanine, 6.0 g serine, 2.5 g histidine, 1.3 g lysine, 1.0 g cysteine, and 46.8 g proline. The ammonium salts and α-amino acids (all amino acids except proline) in the medium were considered to be assimilable nitrogen (Salmon and Barre, 1998).

Medium MS300 contained an equivalent of 324 mg N/L that was assimilable by yeasts (204 mg N/L from amino acid solution corresponding to 13 mL/L of amino acid solution and 120 mg N/L from NH₄Cl corresponding to 0.46 g/L). Medium MS170 contained only 176 mg N/L of assimilable nitrogen (108 mg N/L from amino acid solution corresponding to 7.4 mL/L and 68 mg N/L from NH₄Cl corresponding to 0.26 g/L). The pH of each medium was adjusted to 3.3 before autoclaving for 15 min at 121 °C. Vitamins were filtered and added after the thermal treatment.

3. Analytical methods

3.1. Biomass

Yeast growth was determined by cell counting, using a Thoma haemocytometer. Samples were withdrawn throughout the fermentations and diluted appropriately in 0.9 % (w/v) NaCl solution. Living and dead cells were differentiated by applying a methylene blue staining procedure.

3.2. Sugar, ethanol and glycerol

Samples were filtrated (0.45 μm), diluted ten times and kept at -20 °C until analysis. Metabolite concentrations were determined using high-performance liquid chromatography (Thermo Scientific, France) with a Rezex ROA-Organic acid H⁺ (8 %), 250 × 4.6 mm phase-reverse column (Phenomenex, France). The column was eluted with a degassed mobile phase containing 10 mM sulfuric acid, at 30 °C and at a flow rate of 0.170 mL/min. The injection loop volume was 25 μL. Glucose, fructose, ethanol and glycerol peaks were detected using a refractive index detector (Finnigan Surveyor RI Plus detector, Thermo Scientific, France).

3.3. Nitrogen

Amino acid and ammonium chloride were the two sources of yeast assimilable nitrogen (YAN). Amino acid nitrogen was measured using an o-Phthaldialdehyde/N-Acetyl-L-Cysteine assay (K-PANOPA; Megazyme International Ireland Ltd). Free ammonium salts were measured using an enzymatic assay (K-AMIAR; Megazyme International Ireland Ltd). Taking into consideration ammonium chloride and nitrogen atoms only present in the α-amino position of amino acids, media MS300 and MS170 contained 324 and 176 mg N/L respectively (Taillandier et al., 2014). For stoichiometric purposes, all the nitrogen atoms assimilable by the yeast had to be taken in account, including those not in the α-amino position of amino acids; MS300 and MS170 thereby contained 429 and 243 mg N/L respectively. The measured YAN concentration with only nitrogen in the α-amino position is proportional to YAN concentration containing all nitrogen atoms in amino acids.

3.4 Elementary analysis

The sample was centrifuged for 5 minutes at 10000 rpm. The supernatant was withdrawn and ultrapure water was added to the precipitate. The mixture was then stirred and centrifuged. This washing step was repeated three times. The obtained precipitate was placed in a vacuum oven at 105 °C. Mass fractions of carbon, hydrogen and nitrogen were determined under dry combustion using a PERKIN ELMER 2400 micro analyser (Jimenez and Ladha, 1993).

4. Fermentation

Each fermentation was performed in a 2.5 L reactor filled with 1.5 L of medium. The reactor has a sampling probe and an opening at the top equipped with a filter (0.45 μm) to avoid overpressure. The inoculation volume was calculated to initially reach 5*10⁸ and 10⁹ cells/L, respectively, for S. cerevisiae and
T. delbrueckii. Pure cultures of S. cerevisiae and T. delbrueckii were performed in MS170 and MS300. The duration of fermentation corresponded to the time needed to consume 97% of the sugar initially present. All fermentations were carried out at 20 °C under magnetic agitation (250 rpm) and were performed in duplicate.

5. Statistical analysis

The data are given as mean values (± standard deviation). Statistical analyses were performed using the RStudio programme (Racine, 2012). Following verification of variance homogeneity (Fisher test, \( p > 0.05 \)), one-way analysis of variance (ANOVA) \( (p < 0.05) \) was used to determine statistically significant differences between modalities. In the text, no significant differences are shown between the mean values compared (if \( p \) value is higher than 0.05) or significant differences (if \( p \) value is lower than 0.05).

MODEL FORMULATION

1. Minimal nitrogen and stored nitrogen

A structured model was developed based on nitrogen-based biomass growth. In this model, the nitrogen in the medium \( (N) \) is assimilated by a yeast cell and distributed between two compartments: the constitutive compartment and the storage compartment. The constitutive compartment contains the minimal quantity of nitrogen \( (N_{\text{min}}) \) required to ensure the vital functions of a cell. Apart from the vacuoles, the constitutive compartment corresponds to all the nitrogenous elements of the cytoplasm, the membranes and the contents of the organelles, including free amino acids. Like the constitutive compartment, the storage compartment contributes to cell activity; however, only the storage compartment contains the nitrogen reserves \( (N_s) \) available for growth. The storage compartment corresponds to the nitrogen content of the vacuoles. During fermentation, the size and composition of amino acids in the cytosolic pool is fairly constant, in contrast to those in the vacuolar pool (Huber-Wälchli and Wiemken, 1979; Kitamoto et al., 1988). Therefore, it can be assumed that the amount of nitrogen in the constitutive compartment \( (N_{\text{min}}) \) is invariable and the amount of nitrogen in the storage compartment \( (N_s) \) varies during fermentation. As the cells multiply, the amount of nitrogen required for the formation of the additional constitutive compartment is drawn from the storage compartment of the mother cell. The remaining nitrogen stock is equitably distributed between the daughter cells (Figure 1).  

2. Additional assumptions

Some assumptions were made to describe the evolution of the fermentation. It was assumed that the reactors used were perfectly agitated and that the volumes sampled over time did not disturb the evolution of the fermentation. The model describes the evolution of total sugars and does not differentiate between glucose and fructose. Similarly, the model considers total assimilable nitrogen without discerning between ammonia and amino acids. Ethanol and glycerol production are two reactions which are considered independent and instantaneous and which use sugar as a substrate. There is therefore

![Diagram of nitrogen partition during cell multiplication](image)

**FIGURE 1.** Nitrogen partition during cell multiplication.  
\( N_{s(\text{i+1})} \) represents the amount of nitrogen in the storage compartment before a cell multiplication occurs at time \( t=\text{i} \).
no accumulation of sugars in the cells. For each yeast, molar mass \( M \) and chemical formula \((\text{CH}_x\text{O}_y\text{N}_z)\) vary during fermentation. It is assumed that molar mass \( M' \) and chemical formula \((\text{CH}_x\text{O}_y\text{N}_z')\) of a cell containing an empty storage compartment do not vary. The minimum amount of nitrogen contained in the constituent compartment is the only nitrogen contribution to this molar mass. This molar mass is called the constitutive molar mass.

3. Stoichiometry

The model is based on five reactions: growth \((r1)\), ethanol production \((r2)\), YAN absorption \((r3)\), yeast death \((r4)\), and glycerol production \((r5)\).

\[ \begin{align*}
&\text{(r1)} \quad \text{C}_6\text{H}_{12}\text{O}_6 + g1 \text{N}_2 + g2 \text{CH}_3\text{O}_2\text{N}_z + g3 \text{CO}_2 + g4 \text{H}_2\text{O} \\
&\text{(r2)} \quad \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{C}_2\text{H}_5\text{O}_2 + 2 \text{CO}_2 \\
&\text{(r3)} \quad 1 \text{N} \rightarrow 1 \text{N}_2 \\
&\text{(r4)} \quad \text{CH}_3\text{O}_2\text{N}_z \rightarrow 1 \text{CH}_3\text{O}_2\text{N}_z \quad \text{(alive)} \\
&\text{(r5)} \quad \text{C}_2\text{H}_4\text{O}_6 + 4 \text{H}^+ + 4\text{e}^- \rightarrow 2 \text{C}_2\text{H}_5\text{O}_2
\end{align*} \]

Nitrogen mass balance in growth reaction \((r1)\) allows us to establish a relationship between stoichiometric coefficients \(g1\) and \(g2\) (Eq. (6)):

\[ z' = \frac{g1}{g2} \]

Therefore, the growth reaction rate is written as follows (Eq. (7)):

\[ \text{(r1)} \quad \text{C}_6\text{H}_{12}\text{O}_6 + g1 \text{N}_2 + g2 \text{CH}_3\text{O}_2\text{N}_z + g3 \text{CO}_2 + g4 \text{H}_2\text{O} \]

The molar mass containing the lowest fraction of nitrogen experimentally observed was \( \text{CH}_1.74\text{O}_0.69\text{N}_0.097 \) and \( \text{CH}_1.71\text{O}_0.63\text{N}_0.113 \) for \( S.\text{cerevisiae} \) and \( T.\text{delbrueckii} \) respectively. These values were used to determine the growth reaction stoichiometric coefficients for each yeast (8-9).

\( S.\text{cerevisiae}: \)

\[ \text{(r1)} \quad \text{C}_6\text{H}_{12}\text{O}_6 + g1 \text{N}_2 = 5.525 \text{CH}_3.19\text{O}_{1.04}\text{N}_{0.131} + 0.475 \text{CO}_2 + 1.191 \text{H}_2\text{O} \]

\( T.\text{delbrueckii}: \)

\[ \text{(r1)} \quad \text{C}_6\text{H}_{12}\text{O}_6 + g1 \text{N}_2 = 5.398 \text{CH}_3.12\text{O}_{1.49}\text{N}_{0.129} + 0.602 \text{CO}_2 + 1.386 \text{H}_2\text{O} \]

The stoichiometric coefficient \(g1\) is identified alongside parameters described in the following section.

4. Kinetics

Reaction rates are expressed in mol/L/h. and \( a \) represent the concentration of living yeast cells and the correlation coefficient linking the concentration of living cells to mass concentration respectively. The value of \( a \) is \( 37.6*10^9 \) and \( 71.6*10^9 \) cells/g for \( S.\text{cerevisiae} \) and \( T.\text{delbrueckii} \) respectively. The constitutive yeast molar concentration, is defined by (Eq. (10)):

\[ X_n = \frac{x}{a+M} \]

Microbial growth rate \((r1)\) is assumed to be limited by ethanol \((E)\) and limited by the nitrogen stock \((N_s)\). It is expressed using the following logistic-like equation (Eq. (11)):

\[ r1 = X_n \cdot k_\mu \cdot \left(1 - \frac{x}{x_{\text{max}}} \right) \left(1 + \frac{K_E}{K_E + E} \right) \left(\frac{N_s}{N_s + KN}\right) \]

where, \( K_\mu \) is the growth kinetics constant, \( x_{\text{max}} \) is the maximum living cells concentration, \( K_E \) is the constant of growth inhibition by ethanol and \( K_N \) is the constant of growth limitation by stored nitrogen. Ethanol production rate \((r2)\) is assumed to be limited by ethanol \((E)\) and limited by sugar \((S)\) and nitrogen stock \((N_s)\) (Eq. (12)):

\[ r2 = k_S \cdot X_n \cdot \left(\frac{S}{S + K_S} \right) \left(\frac{K_E}{K_E + E} \right) \left(\frac{N_s}{N_s + KN}\right) \]

The terms \( K_s \), \( K_S \), \( K_E \), and \( K_N \) represent the constants of ethanol production kinetics, ethanol production limitation by sugar, ethanol production inhibition by ethanol and ethanol production limitation by stored nitrogen respectively.

Nitrogen uptake rate is related to the concentration of assimilable nitrogen and the number of cells present in the medium and is described as follows (Eq. (13)):

\[ r3 = k_N \cdot X_n \cdot N \]

Where \( K_N \) is the constant of nitrogen assimilation kinetics. In the death reaction \((r4)\), \( E_{\text{lim}} \) is the mortality coefficient (Eq. (14)).

\[ r4 = X_n \cdot e^{-E_{\text{lim}}/T} \]

Glycerol production \((r5)\) is limited by low sugar concentration. The production of glycerol \((G)\) is coupled to the oxidation of NADH to NAD\(^+\). Regarding the surplus of NADH produced
during the growth of biomass, the production of glycerol contributes to maintaining the redox equilibrium (Nordstöm, 1968). In addition, the production of ethanol is coupled with the oxidation of NADH to NAD$^+$: the more ethanol produced the less glycerol is needed to maintain the redox equilibrium. The rate of glycerol production is described as follows (Eq. (15)):

$$ r_G = K_G \cdot X_{cell} \cdot \frac{S}{S+K_S} \cdot \frac{K_E}{K_E+G} $$

where $K_G$ is the constant of glycerol production kinetics, is the constant of glycerol production limitation by sugar, and is the constant of glycerol production inhibition while ethanol is being produced. The dynamic evolution of each of the six variables of the model is given by the system (A):

$$ \begin{align*}
\frac{dX}{dt} &= (g2 \cdot r1 - r4) \\
\frac{dS}{dt} &= -(r1 + r2 + r5) \\
\frac{dN}{dt} &= -r3 \\
\frac{dN_{s}}{dt} &= (r3 - g1 \cdot r1) \\
\frac{dE}{dt} &= 2 \cdot r2 \\
\frac{dG}{dt} &= r5 
\end{align*} $$

(A)

5. Calculation methods

All calculations were performed using Matlab® 2014a. Integration of the ordinary differential equations system (A) uses the function ode15 of Shampine and Reichelt (1997). Parameter estimation is based on a constrained non-linear multivariable function minimisation. The function to be minimised is the least-square sum expressing the difference between experimental values and calculated values of the five measured variables: concentration of living yeast cells ($X$), sugar ($S$), YAN ($N$), ethanol ($E$) and glycerol ($G$). A Sequential Quadratic Programming (SQP) method was used to minimise the least-square sum. The confidence intervals given for the estimated parameters correspond to a 10 % quasi-invariance region of the least-square sum. The initial value of the specific nitrogen in storage compartment was 0.1 mg/10^9 cells for both yeasts.

**COMPARISON OF THE MODEL WITH EXPERIMENTAL DATA**

Pure cultures of *S. cerevisiae* and *T. delbrueckii* were created in two media with different initial YAN concentrations. The evolution of 5 variables (concentration of living yeast cells ($X$), sugar ($S$), YAN ($N$), ethanol ($E$) and glycerol ($G$))

| Parameters    | *S. cerevisiae* | *T. delbrueckii* | Unit |
|---------------|----------------|-----------------|------|
| $K_n$         | 0.032 ± 0.003  | 0.029 ± 0.005  | 1/h  |
| $X_{max}$     | 305 ± 40       | 604 ± 73       | 10^6 cell/L |
| $K_X$         | (1±1) *10^{-3}  | (5 ± 4) *10^{-4} | mg N/10^9 cells |
| $gI$          | 0.41 ± 0.06    | 0.42 ± 0.03    | (-)   |
| $K_E$         | 20 ± 7         | 12 ± 5         | g/L   |
| $K_S$         | 0.18 ± 0.03    | 0.17 ± 0.02    | 1/h   |
| $K_{ES}$      | 71 ± 66        | 79 ± 17        | g/L   |
| $K_{NS}$      | 102 ± 74       | 33 ± 7         | g/L   |
| $K_{ES}$      | (5 ± 3) *10^{-6} | 0 | mg N/10^9 cells |
| $K_S$         | 6.1 ± 4.0      | 1.0 ± 0.4      | L/mol/h |
| $E_{lim}$     | 155 ± 14       | 145 ± 9        | g/L   |
| $K_0$         | 1.58 ± 1.26    | 1.20 ± 0.98    | 1/h   |
| $K_{SG}$      | 140 ± 124      | 126 ± 117      | g/L   |
| $K_{EG}$      | 1.6 ± 1.4      | 1.2 ± 0.9      | g/L   |

The confidence intervals given for the estimated parameters corresponding to a 10 % quasi-invariance region of the least-square sum.
were assessed for each performed fermentation. The datasets obtained from those experiments were used to identify the kinetic constants and the stoichiometric coefficient $g_1$. The results of this identification process are given in Table 1. Figures 2 and 3 show both experimental data and simulated curves.

1. Yeast growth

Passing from MS170 medium to MS300 medium induced a 77% increase in initial YAN concentration, engendering a slight increase in concentration of S. cerevisiae maximum living cells (from 240 (±10) *10^9 to 281 (±13) *10^9 cells/L). The increase in initial YAN concentration also induced an increase in T. delbrueckii maximum living cells (from 420 (±20) *10^9 to 542 (±53) *10^9 cells/L), which corresponds to an 29% increase, whereas that of S. cerevisiae was 17%.

In terms of population evolution, the model faithfully describes the latency, growth, stationary and decline phases of each culture of S. cerevisiae and T. delbrueckii (Figures 2 and 3). In each fermentation, the profile of the nitrogen in the storage compartment showed an accumulation phase followed by a depletion phase (Figures 2 and 3). In medium MS170, yeast growth stopped when there was no longer enough stored nitrogen for the production of new cells (Figures 2a and 3a). The model suggests that not enough nitrogen was assimilated from this medium to satisfy requirements for reaching the maximal concentration of living cells (a). This is in accordance with the hypothesis of a limiting nitrogen concentration in MS170 for this

![FIGURE 2. S. cerevisiae pure culture fermentations in synthetic media: MS170 (a) and in MS300 (b). Data points are experimental measurements, while solid lines are dynamic model predictions. Vertical bars represent the standard deviations.](image)
specific strain of *S. cerevisiae* and another strain of *T. delbrueckii* (Taillandier et al., 2014). In medium MS300, nitrogen is not limiting and yeast growth stops when is reached.

2. Nitrogen partition

Early on in the performed fermentations, the yeasts assimilated nitrogen and the content of the storage compartment increased and rapidly became higher than that of the constitutive compartment (Figure 4). In the latency and growth phases, the storage compartment of *S. cerevisiae* contained a nitrogen pool which reached a concentration 5 to 20 times higher than that of the constitutive compartment (Figure 4a). Amino acids were accumulated in vacuoles at levels 5 to 40-fold higher than the corresponding cytosolic concentration of *S. cerevisiae* (Ohsumi and Anraku, 1981; Sato et al., 1984; Klionsky, 1990). The hypothesis regarding the association of the storage compartment with vacuoles can therefore be confirmed.

The model showed that the ratio $N_s/N_{\text{min}}$ was always under 2.5 in *T. delbrueckii* fermentations (Figure 4b). This is much lower than ratios calculated in *S. cerevisiae* fermentations. As far as *T. delbrueckii* is concerned, we have not found data about nitrogen partitioning during fermentation in the literature. The modelling therefore gives an estimation of non-accessed data and additional experiments are necessary to determine the reliability of such an estimation.

![Graph](image)

**FIGURE 3.** *T. delbrueckii* pure culture fermentations in synthetic media: MS170 (a) and MS300 (b). Data points are experimental measurements, while solid lines are dynamic model predictions. Vertical bars represent the standard deviations.
The transport of amino acids from cytosol to vacuole is performed through an energy-requiring process (Okorokov et al., 1985). The higher the $N_s/N_{\text{min}}$ ratio, the higher the energy required to maintain the transport of amino acids against the concentration gradient. The $N_s/N_{\text{min}}$ ratios for $S.\ cerevisiae$ and $T.\ delbrueckii$ were revealed to be different. Our model therefore allows us to conclude that the energy requirements for amino acid transport from cytosol to vacuole in $T.\ delbrueckii$ may be different to those of $S.\ cerevisiae$.

3. Nitrogen requirements

In both media, $S.\ cerevisiae$ (Figure 2) and $T.\ delbrueckii$ (Figure 3) completely consumed the YAN initially present in $\approx 55$ h. In each fermentation, the stored nitrogen profile showed an accumulation phase followed by a depletion phase (Figures 2 and 3). In medium MS170, yeast growth stopped when there was no longer enough stored nitrogen for the production of new cells (Figures 2a and 3a). The model suggests that there is not enough nitrogen assimilated in this medium to satisfy requirements for reaching the maximal concentration of living cells. This is in accordance with the hypothesis of a limiting nitrogen concentration in MS170 for this specific strain of $S.\ cerevisiae$ and another strain of $T.\ delbrueckii$ (Taillandier et al., 2014). In medium MS300, nitrogen is not limiting and yeast growth stops when is reached.

The stoichiometric coefficient $g1$ links the consumption of nitrogen in the storage compartment to cell production. Its value is $0.41 \pm 0.06$ and $0.42 \pm 0.03$ respectively for $S.\ cerevisiae$ and $T.\ delbrueckii$. However, the yeast stoichiometric coefficients $g1$ are not significantly different and the maximal concentration of $T.\ delbrueckii$ living cells () is twice as high as that of $S.\ cerevisiae$. This means that twice as much nitrogen is required to produce one $S.\ cerevisiae$ cell than to produce one $T.\ delbrueckii$ cell. However, the maximum
biomass concentration was close to 8 g/L for all fermentations of *S. cerevisiae* and *T. delbrueckii*. Despite both yeasts having a similar maximal dry weight, *T. delbrueckii* performs fermentations with a higher maximal concentration of living cells. The latter observation, alongside the nitrogen requirements suggested by the model, are coherent with the fact that *S. cerevisiae* cells size is higher than that of *T. delbrueckii* (Hernández-López et al., 2007).

The determined yeast constitutive molar mass was CH$_{1.741}$O$_{0.698}$N$_{0.074}$ and CH$_{1.710}$O$_{0.632}$N$_{0.078}$ for *S. cerevisiae* and *T. delbrueckii* respectively. For both yeasts, nitrogen fractions experimentally measured during the fermentations were higher than the estimated nitrogen fraction of yeast with an empty storage compartment (data not shown). The minimum amount of nitrogen required to ensure the vital functions of cells was 38 and 40 mg N per gram of yeast for *S. cerevisiae* and *T. delbrueckii* respectively.

4. Ethanol production

Sugar had been completely consumed by the end of all performed fermentations. For both yeasts, final ethanol concentration was $\approx$105 g/L and it was not significantly affected by initial YAN concentration. By increasing the initial concentration of YAN, the fermentation time decreased from $197 \pm 11$ to $117 \pm 7$ h and from $245 \pm 11$ to $218 \pm 7$ h for *S. cerevisiae* and *T. delbrueckii* respectively. The ethanol production reaction ($r_2$) is limited by sugar and stored nitrogen and is inhibited by ethanol. The evolution of sugar limitation, stored nitrogen limitation and ethanol inhibition are represented as a function of the consumed sugar in figure 5. For both yeasts, sugar limitation and ethanol inhibition continuously occurred while sugar was being consumed. The identified value of was 0 mg N/10$^9$ cells for *T. delbrueckii*. In both media the production of ethanol by *T. delbrueckii* was not limited by stored nitrogen. Therefore, the lower ethanol production rate observed in

![Figure 5](https://example.com/figure5.png)

**FIGURE 5.** Fermentation limitations and inhibition in *T. delbrueckii* (a) and *S. cerevisiae* (b) pure cultures. *T. delbrueckii* stored nitrogen limitation has the same profile in MS170 and MS300 media.
T delbrueckii fermentation in MS170 was caused by the lower concentration of living cells rather than a limitation of stored nitrogen. The model enabled us to rank the effect of limitations and inhibition on ethanol productivity, and therefore to rank these phenomena.

The identified value of was \((5 \pm 3) \times 10^{-6}\) mg N/10^9 cells for S. cerevisiae. In MS300, ethanol production of S. cerevisiae was not limited by stored nitrogen. In MS170, S. cerevisiae consumed the stored nitrogen until there was not enough for the production of new cells. Subsequently, the stored nitrogen suddenly became limiting for ethanol production and the ethanol production rate sharply decreased by 55 % (Figure 5b). Unlike that of T. delbrueckii, the ethanol production rate of S. cerevisiae was impacted by the limited stored nitrogen in MS170.

CONCLUSION AND FUTURE WORK

1. Conclusion

The present comprehensive model of wine-making fermentation is based on the partition of assimilated nitrogen between the constitutive and the storage compartments and includes the following variables: yeast living cells, sugar, YAN, ethanol, glycerol and stored nitrogen. It contains a set of five ordinary differential equations in order to efficiently predict the evolution of S. cerevisiae and T. delbrueckii pure culture fermentations. This mass-balance model provides a stoichiometric approach to biomass production, unlike nitrogen backboned models used in winemaking. The developed model gives an estimation of non-accessed data, such as nitrogen partition between vacuole and cytosol during fermentation. Although this estimation is coherent with the partition of amino acids observed in S. cerevisiae fermentation, additional experiments are necessary to determine its reliability.

2. Future work

The developed model is robust enough to give a precise description of the fermentation evolution of the pure cultures of two yeasts. The next step will be to validate the model with more initial concentrations of yeast assimilable nitrogen. The model will be further developed so that it can be used for modelling the mixed culture fermentation of S. cerevisiae and T. delbrueckii.

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