**Conception of a** **compartmental nitrogen model describing pure cultures of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* in synthetic media.**

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**Highlights**

* A mathematical model was developed to describe pure culture of two oenological yeasts.
* Compartmental nitrogen model enables to accurately describe fermentation kinetics.
* This model was validated in several synthetic media.

**1. Introduction**

In winemaking the use of mixed cultures combining *Saccharomyces* and non-*Saccharomyces* yeasts is more and more common in order to obtain wines with different organoleptic products. However, the behavior of these yeasts is difficult to control because of the interactions existing between them. In particular, the assimilable nitrogen is often a limiting nutrient in the grape musts and the yeasts are going to be in competition for it [1]. Modeling of the fermentation kinetics taking into account this limiting nutrient could allow to predict the kinetics of mixed culture in a second step.

**2. Methods**

Two commercial oenological yeasts were used in this study: *Torulaspora delbrueckii* Zymaflore alpha® and *Saccharomyces cerevisiae* QA23® Pure cultures of each yeast were performed in three synthetic media MS170, MS300 and MS300M at 20°C [2]. Each medium contained the same amount of sugars (220 g/L), vitamins and mineral elements but different concentration of nitrogen and/or lipids [1-2]. Growth, substrates and metabolites kinetics were followed during the fermentation.

**3. Results and discussion**

In all the pure cultures of *S. cerevisiae* and *T. delbrueckii*, it has been observed that yeasts were still growing whereas nitrogen initially present in the medium was completely exhausted (Fig 1). Therefore, after the nitrogen exhaustion the yeasts growth goes on on a nitrogen source located inside the cell. So It is necessary to consider an intracellular nitrogen source to mathematically describe the growth kinetics of the microorganisms studied. We introduce the notions of minimum cellular nitrogen and stored nitrogen to describe population, substrates and metabolites kinetics. This involves the design of a structured model in which the total assimilated nitrogen is divided into two compartments: the constitutive compartment and the storage compartment.

The constitutive compartment contains a quantity of nitrogen equal to the minimum amount of cellular nitrogen (Nmin). This compartment provides vital minimal functions to keep the cell alive. Like the constitutive compartment, the storage compartment contributes to cell activity. However, only the storage compartment contains the nitrogen reserves available for growth.

The measured molar mass of the yeasts (CHxOyNz) varies during fermentation. The molar mass CHxOyNz' of a cell containing an empty nitrogen storage compartment was considered constant. The latter molar mass let to propose a stoichiometric growth reaction (r1) for each yeast:

1 C6H12O6 + g1 NS 🡺 g2 CHxOyNz' + g3 CO2 + g4 H2O

The evolution of substrates (sugar (S) and nitrogen (N)) and products (ethanol (E) and glycerol (G)) was described using additional stoichiometric reactions such as fermentation (r2), nitrogen absorption (r3), yeast mortality (r4) and glycerol production (r5):

X and Xn represent the living cell expressed respectively in cells/mL and mol/L. The model accurately describes the evolution of the fermentation (Fig 1 and 2). Modification of this model enables to describe precisely effects of lipid supplementation on yeast behavior (data not shown).

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| **Figure 1.** Pure culture of *S. cerevisiae* in MS170.🞨Medium nitrogen (exp) 🞍🞍 Medium nitrogen (model) ⚫ Living cells (exp) ▬ Living cells (model) ▬ ▬Stored nitrogen (model). | **Figure 2.** Pure culture of *S. cerevisiae* in MS170.◊Glycerol (exp) 🞍🞍 Glycerol (model) 🞨Sugar (exp) ▬ ▬ Sugar (model) ▲Ethanol (exp) ▬ Ethanol (model). |

**4. Conclusions**

The proposed compartmental nitrogen model enables to accurately describe growth, substrates and metabolites kinetics during fermentation. That indicates the pertinence of the hypothesis made and constitutes a first step before modeling mixed culture fermentation.

**References**

1. P. Taillandier, Q.P. Lai, A. Julien-Ortiz, C. Brandam. World J Microbiol Biotechnol 30 (2014) 1959–1967.
2. P. Brou, P. Taillandier, S. Beaufort, C. Brandam. Eur Food Res Technol (2018)