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Influence of Oxygen on Alcoholic Fermentation by a Wine Strain of *Torulaspora delbrueckii*: Kinetics and Carbon Mass Balance

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***Torulaspora delbrueckii* metabolism was assessed in a synthetic culture medium similar to grape must under various conditions: no aeration and three different oxygen feeds, in order to determine the effect of oxygen on metabolism. Carbon and nitrogen mass balances were calculated to quantify metabolic fluxes. The effect of oxygen was to decrease the flux of carbon going into the fermentation pathway in favor of growth. In the absence of aeration, higher amounts of glycerol were produced, probably to maintain the redox balance. The oxygen requirement of this strain was high, since even for the highest air supply oxygen became limiting after 24 h. Nevertheless, this strain developed well in the absence of oxygen and consumed 220 g/L of sugars (glucose/fructose) in 166 h at 20 °C, giving a good ethanol yield (0.50 g/g).**

Key words: *Torulaspora delbrueckii*; oxygen; alcoholic fermentation; wine; carbon balance

In the wine production process, the step of alcoholic fermentation (AF) is essential to the final result. The choice of active yeast strain that transforms sugars into ethanol is an especially important factor. Traditionally, yeasts belonging to the *Saccharomyces* genus are used, but an increasing number of studies of non-*Saccharomyces* fermentation yeasts reflect the current interest in the use of these microorganisms in oenology.^{1–3} Among non-*Saccharomyces* yeasts, the most studied are *Pichia*, *Hanseniaspora*, *Kluyveromyces*, *Candida*, *Kloeckera*, and *Torulaspora*.^{4–6} These yeasts are used in addition to the traditional *Saccharomyces* ones. The objective is to couple the fermentative efficacy of *Saccharomyces* with the specificities of the non-*Saccharomyces*, in particular for the production of typical aroma and the development of specific organoleptic qualities. Of these non-*Saccharomyces* yeasts, it has been found that *Torulaspora* is notably productive of positive volatile compounds,

including ethyl butanoate, hexanoate, octanoate, and decanoate.^{7–9} It has also been found that *T. delbrueckii* strains produce smaller amounts of negative aroma compounds, such as acetaldehyde and acetoin, and reduce volatile acidity.^{1,10} Hence *T. delbrueckii* is globally considered a yeast strain giving fruity notes to wines.² However, a major problem occurs with the use of *T. delbrueckii*, since it may present difficulties in growing significantly under winemaking conditions, even when it is inoculated, due to competition with *S. cerevisiae* strains and other indigenous yeasts.¹¹

Before studying the interaction phenomena among these strains, it is important to determine the behavior of *T. delbrueckii* in pure culture, and particularly influence of oxygenation on *T. delbrueckii* metabolism during AF under winemaking conditions. A study by Alves-Araujo *et al.*¹² found that the oxygen requirements of *T. delbrueckii* were higher than those of an *S. cerevisiae* strain, but the study was done under bread fermentation conditions with *T. delbrueckii* isolated from wheat. A study by Hanl *et al.*¹³ showed that oxygen was of prime importance for the metabolism of a *T. delbrueckii* strain but again the culture medium was different from the traditional AF of winemaking, since it was a chemostat culture in 30 g/L of YPD. In some cases, it has been reported that *T. delbrueckii* does not grow under anaerobic conditions.^{14–16} In a variety of studies, it is also classified as a Crabtree-positive^{12,17} or a Crabtree-negative species.¹⁸

The objective of this study was to test and quantify the influence of oxygenation on the metabolism of one *T. delbrueckii* strain selected for wine production. The study was performed in batch fermentors with synthetic grape must. The aeration feed rates tested were made higher than the oxygen quantities usually encountered in winemaking in order to accentuate the phenomena and to gain a good understanding of the effects of this parameter on *T. delbrueckii* metabolism.

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Abbreviations: A_{12} and A_{21} , binary interaction parameters ($A_{12} = 1.7392$ and $A_{21} = 0.9253$); D_E , gas inlet rate of the reactor (L/h); D_S , gas outlet rate of the reactor (L/h); P , atmospheric pressure (760 mm Hg); $P_{\text{eth}}^{\text{O}}$ (T), pressure of saturated vapor of ethanol at temperature T; O_2 , dissolved oxygen concentration in the medium (mg/L); O_2^* , saturated dissolved oxygen concentration in the medium (8.8 mg/L at 20 °C in water); r_{O_2} , rate of oxygen consumption (mol/L); r_{CO_2} , rate of CO₂ production (mol/L); x_{eth} , molar fraction of ethanol in liquid phase; $X_E^{N_2}$, molar fraction of N₂ at the inlet of the reactor (air); $X_E^{O_2}$, molar fraction of O₂ at the inlet of the reactor; $X_E^{CO_2}$, molar fraction of CO₂ at the inlet of the reactor; $X_S^{O_2}$, molar fraction of O₂ at the outlet of the reactor; $X_S^{CO_2}$, molar fraction of CO₂ at the outlet of the reactor; X_S^{eth} , molar fraction of ethanol at the outlet of the reactor as calculated by a mathematical model; VM, molar volume of perfect gas at 20 °C at 1 bar (24.534 L/mol); y_{eth} , molar fraction of ethanol in gas phase; γ_{eth} , activity coefficient of ethanol

Materials and Methods

Yeasts and medium. The microorganism used was *Torulaspora delbrueckii* NSC123, commercialized by Lalemand (Montreal, Canada) for alcoholic fermentation in winemaking. The composition of the synthetic medium used in this study was designed to be close to that of white grape must and to avoid limitations on carbon, nitrogen, vitamins, and mineral elements needed for yeast growth. It was a medium regularly used in oenological studies, MS300. The composition was glucose (110 g/L), fructose (110 g/L), malic acid (6 g/L), citric acid (6 g/L), KH_2PO_4 (0.75 g/L), K_2SO_4 (0.5 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.25 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.16 g/L), NaCl (0.2 g/L), anaerobic factor, oligoelement, and vitamin solutions. The details of these composition solutions are given in Bely *et al.*¹⁹ The nitrogen was brought by ammonium ions in NH_4Cl and by a mixture of amino acid solution. Medium MS300 contained the equivalent of 324 mgN/L assimilable by yeasts (204 mg N/L corresponding to 13 mL/L of amino acid solution and 120 mg N/L from NH_4Cl corresponding to 0.46 g/L). The pH of the medium was adjusted to 3.3 with a sodium hydroxide solution (10 N) before autoclaving for 20 min at 120 °C. Vitamins and oligoelements were added after thermal treatment.

Fermentation experiments. Fermentation was carried out in a 1.8 L New Brunswick Scientific (NBS) reactor with agitation at 250 rpm. Temperature was set at 20 °C. For all rounds fermentation, initial inoculation was fixed at 10×10^6 viable cells/mL of *T. delbrueckii*. Samples of 20 mL were regularly withdrawn and centrifuged at 10,000 rpm for 10 min at 0 °C. The supernatants obtained were stored at -20 °C for further analysis. The criterion for the end of AF was set at the time at which 95% of initial are consumed, which corresponds to 10 g/L of residual sugars. In order to determine the effect of oxygen on the AF of *T. delbrueckii* NSC123, oxygen supplies for fermentation were regulated by mass flowmeters under four conditions: 0 mL/min, 540 mL/min, 1,080 mL/min, and 1,800 mL/min of air. These aeration flows were called 0, 0.3, 0.6, and 1 VVM (volume of air per volume of medium per min) respectively. In the 0 VVM experiment, dissolved oxygen was present initially in liquid phase (9 mg/L), but during the remainder of fermentation no more oxygen was supplied as in wine fermentation. Each experiment was performed in triplicate. The values of the oxygen transfer coefficient between the gas and the liquid phase (K_La) were determined to be 8.0, 13.1, and 19.9 h⁻¹ for aeration rates of 0.3, 0.6, and 1 VVM respectively.

Compound analysis. The yeast population was followed by observing the optical density (OD) of the yeast suspension at 620 nm. Using data from specific experiments, a linear correlation (for OD < 0.8) between the dry weight of biomass, measured by thermo-balance using infrared rays, and the optical density $\text{OD}_{620\text{nm}}$ was determined as follows: $X_{\text{biomass}} \text{ (g/L)} = 1.1353 \times \text{OD}_{620\text{nm}}$ ($R^2 = 0.9613$).

The elemental composition of the biomass was measured by CHNS/O AE1110 and Flash-EA1112 analyser (Thermo Finnigan, LGC, Toulouse, France). The principle was to use chromatography to analyze the gases produced by the combustion of a solid biomass sample in oxygen at 1,800 °C. The analyzer gave the composition in C, H, O, N.

Glucose was analyzed by an enzymatic method performed automatically by a YSI 2700 instrument. The total sugar amounts were determined using dinitrosalicylic reagent, as described by Miller.²⁰ The difference between total sugars and the glucose concentration gave the fructose concentration. The concentrations of ammonium, L-alpha amino, acid nitrogen, L-malic acid, L-lactic acid, and acetic acid were analysed by specific enzymatic methods (kits from MicroDOM, LGC, Toulouse, France). The ethanol and glycerol concentrations were measured by HPLC. The apparatus (TSP Spectra System, LGC, Toulouse, France) has a specific column (Biorad Aminex HXP-87H) to separate alcohols, organic acids, and sugars. The liquid phase consists of a 5 mm sulphuric acid solution circulating at 0.4 mL/min. The temperature of the column was set to 40 °C, and the volume of the injection loop was 20 µL. Components were detected using a differential refractometer (TSP RefractoMonitor V, LGC, Toulouse, France).

Dissolved oxygen concentrations in the medium were measured using an oxygen meter with probe. The percentages of O₂ and CO₂

exhaust gas were determined by gas analyzer (Servomex 4100, a paramagnetic transducer for O₂ and an infrared transducer for CO₂).

Calculation of ethanol evaporation. Fermentation done with air flow induced significant evaporation over time. From 1.8 L of medium at the initial time, without any samples, the liquid volumes were only 1.8, 1.75, 1.7, and 1.65 L at the final time for fermentation at 0, 0.3, 0.6, and 1 VVM respectively. The evaporated volume was composed of volatile compounds of the medium. We assumed that water and ethanol made up most of these compounds. Other volatile compounds were certainly also present in the evaporated volume, but their relatively small quantities were assumed to be negligible compared to those of the two major compounds. In gas, ethanol was not measured but rather calculated by means of a mathematical model. The hypothesis underlying this model was that the liquid medium and gas phase are always in thermodynamic equilibrium at the temperature of the condenser present in the exit gases (18 °C). For the equilibrium calculation, the medium was assimilated to a binary mixture of water and ethanol. The equilibrium equation giving the ethanol concentration in gas, y_{eth} is as follows:

$$y_{\text{eth}} = \gamma_{\text{eth}} \cdot x_{\text{eth}} \cdot \frac{P_{\text{eth}}^0(T)}{p}$$

The activity coefficient of ethanol (γ_{eth}) was calculated by a model of the activity coefficient. For this classical binary system, a simple model such as the Margules law can be used:

$$\gamma_{\text{eth}} = \exp \left[\frac{A_{12}}{1 + \frac{A_{12}x_{\text{eth}}}{A_{12}(1-x_{\text{eth}})}} \right]$$

With this model the ethanol concentration in the gas phase was calculated and, the flow of the gas being known for each experiment, it was possible to determine the quantity of ethanol evaporated and to rebuild the global quantity of ethanol produced (ethanol in liquid phase + ethanol in gas phase). In order to validate this ethanol evaporation model, experiments were performed with only ethanol-water solutions of 90 g/L in an NBS reactor, with aeration at 0, 0.3, 0.6, and 1 VVM. The ethanol concentrations in the liquid phase were measured over time. At 150 h, they had decreased from 90 g/L to 55 g/L, 35 g/L, and 18 g/L for the three patterns air flow and they remained constant for 0 VVM. Evaporation is the only explanation for this decrease. As Fig. 1 shows, the model fit the experimental data very well.

CO₂ production and O₂ consumption. CO₂ and O₂ withdrawn from the AF were analyzed and recorded in real time by automatic gas analyzer (CO₂/O₂), connected to the NBS reactor. The data were processed to calculate the accumulated values of CO₂ production and O₂ consumption by integrating the rates of O₂ consumption, r_{O_2} , and CO₂ production, r_{CO_2} , over time.

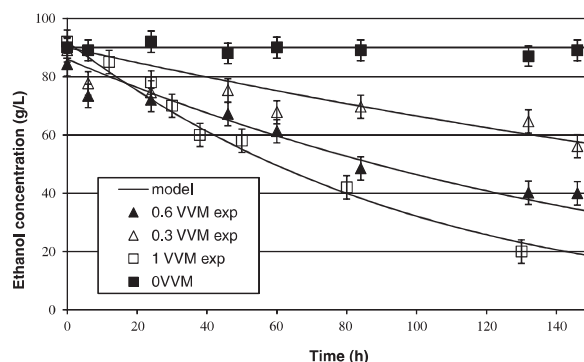


Fig. 1. Comparison of Experimental and Modelled Values for Ethanol Evaporation in Aerated Fermentor at Oxygen Flows of 0, 0.3, 0.6, and 1 VVM.

Experiments performed in a 1.8 L reactor, agitation at 250 rpm, 20 °C in a water-ethanol medium (90 g/L).

$$r_{O_2} = \frac{(D_E^* X_E^{O_2}) - (D_S^* X_S^{O_2})}{VM}$$

$$r_{CO_2} = \frac{(D_S^* X_S^{CO_2}) - (D_E^* X_E^{CO_2})}{VM}$$

with

$$D_S = \frac{(D_E^* X_E^{N_2})}{1 - (X_S^{O_2} + X_S^{CO_2} + X_S^{eth})}$$

Distribution of carbon between fermentation, respiration + biomass, and glycerol production pathways. According to the AF reaction, 1 mole of CO₂ is degassed for each mole of ethanol produced: Glucose → 2 Ethanol + 2CO₂.

Assuming that all of the ethanol produced comes from this reaction, it is possible to calculate the quantity of CO₂ produced by the reaction. The difference between the total measured CO₂ and the calculated CO₂ thus corresponds to the CO₂ produced by other metabolic pathways. Another, non-negligible, metabolic pathway for the carbon consumed in the AF is the production of glycerol. It is thus possible to calculate the proportion of carbon consumption that goes into each of the pathways:

- Fermentation: carbon of the ethanol and CO₂ produced by the AF
- Glycerol: carbon of the glycerol produced
- Respiration + biomass production: carbon of the biomass produced plus the carbon of CO₂ produced in other ways than by AF (essentially by respiration).

Results

Influence of aeration on the distribution of the carbon in the various pathways

All of the main carbon compounds were measured at the beginning and the end of the AF (considered as the time when the residual sugar was 10 g/L). Table 1 shows the final concentrations of the principal carbon compounds for the four experiments.

By focusing on the ethanol production of these four rounds of fermentation at 0, 0.3, 0.6, and 1 VVM, we noted that the amounts of ethanol produced were very close whatever the amount of aeration: 104.6, 101, 98.1, and 99.9 g/L respectively. However, the trend was towards lower production of ethanol with increasing aeration, and consequently the ethanol yields from the consumed sugars (210 g/L) decreased: 0.500, 0.483, 0.469, and 0.464 g/g with rising aeration rates.

The amounts of biomass formed were also dependent on aeration. Higher aeration rates increased the amount

of biomass. Thus 4.4 g/L of biomass was produced during the AF at 0 VVM, 8.7 g/L at 0.3 VVM, 9.5 g/L at 0.6 VVM, and 10.8 g/L at 1 VVM. Oxygen consumption and carbon dioxide production followed the same trend from 0 g/L to 24.1 g/L (0.75 mol/L) of oxygen consumed and 99.9 to 129.4 g/L (2.27 to 2.94 mol/L) for CO₂ produced by AF for 0 to 1 VVM.

The quantities of CO₂ produced otherwise than by AF corresponded almost exactly to the quantities of oxygen consumed. The relation between the CO₂ produced by other metabolism pathways and the oxygen consumed (the respiratory quotient) varied within a range 0.97 to 1.04 here, and thus was close to 1. This is consistent with what is usually described for the respiratory chain of yeasts causing the production of biomass. As regards glycerol production, the trend was inverted relative to biomass production. Glycerol was produced in greater quantities when the AF was not aerated: 13.4 g/L at 0 VVM as against 2.5 g/L at 1 VVM.

For the organic acids measured, few significant differences were observed in the production of lactic, acetic, and succinic acid. Citric acid was neither consumed nor produced, since the initial concentration of 6 g/L was found again at the end of the AF, whatever the aeration. In contrast, malic acid was consumed in greater quantities when aeration was higher: 3.5 g/L at 1 VVM as compared to 1.1 g/L at 0 VVM.

The atomic composition in C, H, O, N, of the *T. delbrueckii* dried biomass was analyzed at the beginning and the end of each aeration flow condition. Table 2 presents these results. This composition varied depending on the conditions of aeration. For AF in the absence of aeration (0 VVM), the nitrogen fraction was higher and the fraction of oxygen lower. The nitrogen concentration was 6.5% and that of oxygen was 37% for this AF, against about 3% and 40% respectively for the other three rounds of fermentation. The initial biomass nitrogen and oxygen content were intermediate between the non-aerated modality and the aerated ones.

Taking into account the percentages of carbon in the chemical compositions of these components and correcting for the variation in volume due to evaporation during AF, it was possible to establish the carbon balance. The results indicated that C was conserved

Table 1. Principal Characteristics of Fermentation with Aeration at 0, 0.3, 0.6, and 1 VVM

	0 VVM	0.3 VVM	0.6 VVM	1 VVM
End of fermentation (h)	166 ± 10	132 ± 10	142 ± 5	137 ± 4
Biomass produced (g/L)	4.4 ± 0.36	8.7 ± 0.56	9.5 ± 0.75	10.8 ± 0.26
Final cell concentration (10 ⁶ /mL)	430	870	1010	1380
Ethanol produced (g/L)	104.6 ± 2.4	101.0 ± 1.1	98.1 ± 2.5	97.0 ± 1.7
Glycerol produced (g/L)	13.4 ± 1.6	6.3 ± 0.6	2.2 ± 0.5	2.5 ± 0.2
Acetic acid produced (g/L)	0.31 ± 0.02	0.25 ± 0.04	0.28 ± 0.02	0.30 ± 0.02
Lactic acid produced (g/L)	0.11 ± 0.02	0.14 ± 0.01	0.10 ± 0.02	0.11 ± 0.02
Succinic acid produced (g/L)	0.31 ± 0.06	1.7 ± 0.10	1.7 ± 0.05	0.4 ± 0.02
CO ₂ produced by AF (mol/L)	2.27 ± 0.13	2.19 ± 0.12	2.13 ± 0.06	2.17 ± 0.08
CO ₂ produced in other ways (mol/L)	0.00 ± 0.01	0.28 ± 0.02	0.53 ± 0.04	0.77 ± 0.05
Malic acid consumed (g/L)	1.1 ± 0.3	2.6 ± 0.2	3.6 ± 0.3	3.5 ± 0.3
Citric acid consumed (g/L)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.1
NH ₄ ⁺ consumed (mg/L)	120 ± 0.0	120 ± 0.0	120 ± 0.0	120 ± 0.0
N _α consumed (mg/L)	184.5 ± 3.0	192.5 ± 2.5	193.5 ± 1.8	191.8 ± 2.0
O ₂ consumed (mol/L)	0.00 ± 0.02	0.29 ± 0.03	0.51 ± 0.02	0.75 ± 0.04
Ratio (CO ₂ produced in other ways/O ₂ consumed)	/	0.97	1.04	1.02
Yield of ethanol/sugars (g/g)	0.500 ± 0.01	0.483 ± 0.01	0.469 ± 0.01	0.464 ± 0.01

Table 2. Elemental Composition of *T. delbrueckii* Dried Biomass at the Beginning and the End of Fermentation with Aeration at 0, 0.3, 0.6, and 1 VVM

	Oxygen (%)	Carbon (%)	Nitrogen (%)	Hydrogen (%)	Total (%)
Beginning of experiments	38.8	44.2	4.6	7.0	94.6
End of 1 VVM experiment	40.1	44.2	3.0	6.9	94.1
End of 0.6 VVM experiment	39.8	43.7	3.1	6.9	93.4
End of 0.3 VVM experiment	40.4	45.3	3.3	7.2	96.2
End of 0 VVM experiment	37.8	43.3	6.5	6.7	94.3

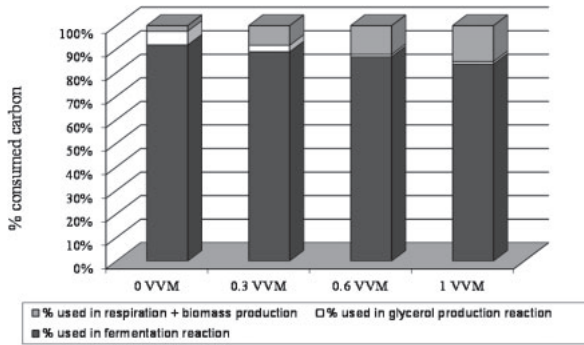


Fig. 2. Distribution of Consumed Carbon between the Metabolism Pathways of AF, Production of Glycerol and Respiration Chain for Four Conditions of Aeration.

between the initial and final times, since the differences between the initial and the final carbon were 0.2%, 0.2%, 0.1%, and 2.7%, respectively for fermentation at 0, 0.3, 0.6, and 1 VVM. These percentages are very low given the experimental uncertainties in sampling and measurement, and show good consistency with the experimental values. Also, this confirms that no other major carbon constituent was produced during AF.

Finally, Fig. 2 recapitulates the distribution of carbon consumption among the three main pathways: alcoholic fermentation, respiration + biomass production, and glycerol production for the four aeration conditions. The effect of oxygen appears clearly. Its consumption in AF passed from 92% to 84%, in respiration + biomass production from 2% to 15% and in glycerol production from 6% to 1% for aeration at 0 VVM and 1 VVM respectively.

Influence of aeration on nitrogen compounds

In the same manner as for the carbon balance, based on the measurements of the ammonium, assimilable α -amino nitrogen and concentrations of the biomass, knowing the chemical composition in nitrogen, it was possible to establish the nitrogen balance. These measurements allowed us to evaluate the global quantity of nitrogen compounds at the beginning and at the end of the AF. Despite experimental errors, the percentage differences obtained were also acceptable: 0.7, 3.2, 1.5, and 9.7%. That allowed us to confirm the coherence of our experimental results and to conclude that no nitrogen was present in any of the other major constituents.

The quantity of nitrogen consumed was almost identical for the four experiments. Ammonium was totally exhausted and there remained only 10 mg/L to 20 mg/L of assimilable nitrogen at the end of the AF (Table 1). All the nitrogen consumed was found in the

biomass of *T. delbrueckii*. It is interesting to note that a similar quantity of nitrogen was consumed to form different quantities of biomass (4.4 g/L to 10.8 g/L). In fact, this difference can be found again in the proportions of nitrogen present in the chemical formula of the *T. delbrueckii* dried biomass at the end of the AF (Table 2). Everything happened as if the nitrogen present in the medium was always stored in the cells. According to the conditions of aeration, the yeast cells drew more or less nitrogen into their stock to form the biomass.

Influence of aeration on fermentation kinetics

An effect of oxygen on the fermentation kinetics was observed. As the first line of Table 1 shows, the AF durations for the four aeration rates, 0, 0.3, 0.6, and 1 VVM, were 166 h, 132 h, 142 h, and 137 h respectively. The three aerated thus had fairly similar AF durations (10 h of variation), whereas the non-aerated AF had a duration that was clearly longer (24 h to 34 h longer) than that of the aerated ones.

These observations on AF durations can be made again for glucose consumption: little difference in glucose consumption rates between aerated, but slower consumption for the non-aerated fermentation (Fig. 3A). Fructose showed the opposite trend, the differences of consumption being considerably larger with aeration (Fig. 3B). The greater the aeration was, the higher was the fructose consumption rate. When aeration was present (0.3, 0.6, or 1 VVM), this *T. delbrueckii* strain consumed glucose in preference to fructose, and all of the glucose was consumed in about 110 h when the medium was aerated, whereas some fructose remained (10 g/L) after 140 h. In contrast, when the AF was not aerated, glucose and fructose were consumed at equal rates, and after 166 h, the same amounts of glucose and fructose remained (5 g/L). The calculation of mean sugar consumption rates confirmed this phenomenon: 1.1 g of glucose consumed per L per h and 0.85 g of fructose consumed per L per h for aerated fermentation, and 0.63 and 0.7 g/L/h respectively for the non-aerated AF.

For the four conditions of fermentation, the amount of soluble oxygen in the liquid medium was zero as early as at 24 h of AF (data not shown). Whatever the aeration level, all the oxygen transferred from the gas phase to the liquid was immediately consumed by this *T. delbrueckii* strain. The rate of oxygen consumption corresponded to the rate of oxygen transfer from the gas to the liquid phase, which is governed by the following equation:

$$\frac{d[O_2]}{dt} = KLa \cdot (O_2^* - O_2)$$

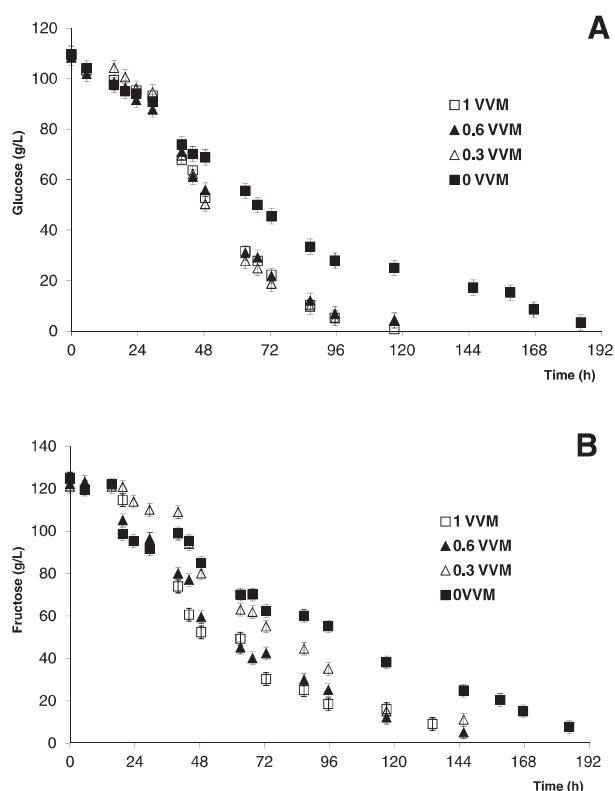


Fig. 3. Consumption of Glucose (A) and of Fructose (B) during Fermentation with Aeration at 0, 0.3, 0.6, and 1 VVM.

As the oxygen concentration in the liquid phase was zero after 24 h of AF, this equation became

$$\frac{d[O_2]}{dt} = K_{la} \cdot (O_2^*)$$

which corresponds to a constant rate, and a linear evolution of cumulated oxygen consumption (curves not shown). Thus these consumptions did not reflect the oxygen requirements of *T. delbrueckii*, but represented the oxygen transfer rate in the medium. For the three forms of aeration, the transfer rates were 70, 115, and 175 mg O₂/L/h respectively for 0.3, 0.6, and 1 VVM. We conclude that *T. delbrueckii* had a maximum oxygen consumption rate higher than the oxygen transfer rate in the medium, one thus greater than 175 mg O₂/L/h. At the moment where oxygen became limiting in the medium, the biomass concentration was 4 g/L, and hence the specific oxygen consumption rate was 1.36 mmol O₂/g/h.

Discussion

Fermentation carried out by varying the aeration between 0 VVM and 1 VVM showed that this strain was sensitive to the quantity of oxygen present in the medium. Two types of metabolism took place in this yeast in the presence or absence of oxygen.

In the absence of oxygen, glycerol production of *T. delbrueckii* was favoured, and represented 6% of the carbon flux. This value is comparable to the data available for *S. cerevisiae* under the same conditions, where the carbon flux passed into glycerol is about 5%.⁶⁾ Glycerol production involves the oxidation of one NADH to NAD⁺. During growth, some intermediates

of glycolysis figure in the biosynthesis of cell components, which causes excess of NADH and leads to a redox imbalance. The synthesis of glycerol helps to regenerate NAD⁺ in the cytosol given the initial lack of alcohol dehydrogenase.²¹⁾ This may take place in this *T. delbrueckii* strain that produces large quantities of glycerol under anaerobic conditions. This phenomenon was not observed at the same magnitude in all the strains of *T. delbrueckii* used by Renault *et al.*²²⁾ since they observed lower glycerol production, of about 4 g/L, for 16 strains grown under conditions similar to ours. Another explanation for glycerol accumulation is activation of the expression of GPD1 due to high osmotic pressure, since cytoplasmic glycerol helps to cope with it.²³⁾ In our case the concentration of glucose and fructose in the medium was 220 g/L. *Torulaspora* yeast is known to tolerate osmotic pressure,¹²⁾ and this might be linked with its ability to produce large amounts of glycerol.

In presence of oxygen, glycerol production was much lower. The presence of oxygen allowed the respiratory chain to function by regenerating NAD⁺ and producing more energy. The Krebs cycle could then operate by releasing additional CO₂. In this case, where respiration was activated, larger amounts of CO₂ were produced than in fermentation pathway. The same amount of oxygen was consumed, with the ratio additional CO₂ produced/O₂ consumed near to 1. The large amount of available energy was then used by the yeast cells to produce biomass, which explains the difference from non-aerated AF.

According to the literature, this non-*Saccharomyces* yeast is classified among the Crabtree-negative¹⁸⁾ or Crabtree-positive organisms, but with less sensitivity than *Saccharomyces* according to Alves-Araujo *et al.*¹²⁾ and Merico *et al.*¹⁷⁾ These authors explain that even under limited oxygen conditions, the yield of biomass production of *T. delbrueckii* remains higher than that of *S. cerevisiae* due to the greater proportion of carbon that can flow in the respiratory metabolism. Our results are in accord with these last authors.

The specific consumption rate of *T. delbrueckii*, calculated at 1.36 mmol O₂/g/h for respiro-fermentative conditions, can be compared with those in the literature: Hanl *et al.*¹³⁾ found that for a strain of *Torulaspora* grown continuously at a rate of 0.1 h⁻¹, the minimum oxygen needed to avoid washing was 0.1 mmol/g/h, while the consumption needed to reach 100% respiratory metabolism was 2.6 mmol/g/h. Thus our strain appears to fall within the same range in terms of oxygen requirements. However, differences observed among the three aeration flows, in both metabolism and kinetics, were low. The extra oxygen consumed by this non-*Saccharomyces* strain under high air flow conditions did not enable AF to take place much faster, as would be expected, since the total produced biomass was increased. Several authors (Visser *et al.*¹⁶⁾ and Mauricio *et al.*¹⁴⁾) have that some strains of *T. delbrueckii* do not grow under limited oxygen. The conclusions are different for the strain tested in the present study. This strain developed and showed good AF performance 0 VVM conditions, even though the time of AF was a little longer. In fact, for this non-aerated AF, the amount of biomass produced was far lower than for aerated

fermentations but the sugar consumption rate was partly offset by a higher specific sugar consumption rate.

The performances of our *Torulasporea* strain were comparable in terms of ethanol/sugar yield to those of a strain of *S. cerevisiae* QA23 grown under the same conditions without aeration¹⁸) (0.50 g/g compared with 0.49 g/g). On the other hand, the AF time for this non-*Saccharomyces* (166 h) was longer than that for their *Saccharomyces* (100 h).

The objective in using non-*Saccharomyces* in complement to *Saccharomyces* yeasts during AF for enhancement of the aromatic quality of wines has helped to increase our knowledge of their fermentative potential in a grape must. In the present study, the influence of oxygen was studied on one oenological strain of *T. delbrueckii*. Compared to other strains of *Torulasporea* tested by various authors,^{12,24}) this strain appears to be a good candidate to carry out AF, because it showed good resistance to ethanol, and good fermentative qualities and developed in the absence of significant amounts of oxygen.

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