# Effect of temperature on *Brettanomyces bruxellensis*: metabolic and kinetic aspects

**Cédric Brandam, Claudia Castro-Martínez, Marie-Line Délia, Felipe Ramón-Portugal, and Pierre Strehaiano**

**Abstract:** The effect of temperatures ranging from 15 to 35 °C on a culture of *Brettanomyces bruxellensis* was investigated in regards to thermodynamics, metabolism, and kinetics. In this temperature range, we observed an increase in growth and production rates. The growth behavior was well represented using the Arrhenius model, and an apparent activation energy of 16.61 kcal/mol was estimated. A stuck fermentation was observed at 35 °C as represented by high cell death. The carbon balance established that temperature had no effect on repartition of the glucose consumption between biomass and products. Hence, the same biomass concentration was obtained for all temperatures, except at 35 °C. Moreover, using logistic and Luedeking–Piret models, we demonstrated that production rates of ethanol and acetic acid were partially growth associated. Parameters associated with growth ($\alpha_{eth}$ and $\alpha_{aa}$) remained constant with changing temperature, whereas, parameters associated with the population ($\beta_{eth}$ and $\beta_{aa}$) varied. Optimal values were obtained at 32 °C for ethanol and at 25 °C for acetic acid.

*Key words: Brettanomyces*, temperature, carbon balance, growth and product kinetics, model.

**Résumé :** L'effet de la température sur une culture de *Brettanomyces bruxellensis* a été examiné. L'influence de températures allant de 15 à 35 °C a été étudiée relativement à la thermodynamique, au métabolisme et à la cinétique. Nous avons observé une augmentation des taux de croissance et de production entre 15 et 32 °C. La croissance suivait adéquatement un modèle d'Arrhenius et l'énergie d'activation apparente a été estimée à 16,61 kcal/mol. Un arrêt de la fermentation a été observé à 35 °C à cause d'une forte mortalité cellulaire. La balance en carbone a révélé que la température n'avait pas d'effet sur la répartition de la consommation de glucose entre la biomasse et les produits. Ainsi, la même concentration de biomasse a été obtenue à toutes les températures, sauf à 35 °C. Qui plus est, à l'aide des modèles logistique et de Luedeking–Piret, nous avons démontré que les taux de production d'éthanol et d'acide acétique étaient partiellement associés à la croissance. Les paramètres associés à la croissance ($\alpha_{eth}$ et $\alpha_{aa}$) étaient constants en fonction de la température alors que les paramètres associés à la population ($\beta_{eth}$ et $\beta_{aa}$) variaient. La température optimale à la production d'éthanol était de 32 °C alors qu'elle était de 25 °C pour l'acide acétique.

*Mots-clés : Brettanomyces*, température, balance en carbone, cinétique de croissance et de production, modèle.

[Traduit par la Rédaction]

## Introduction

Yeasts belonging to the genus *Brettanomyces* (the anamorph of *Dekkera*) have long been recognized as contaminants of wine (Kunkee and Amerine 1970; Larue et al. 1991; Chatonnet et al. 1995). In winemaking, this yeast appears at post-fermentation and can produce unpleasant odors such as "mousy taint" and "horse sweat taste," which deeply affect the wine aroma (Heresztyn 1986; Fugelsang 1997). In industrial alcoholic fermentation, *Brettanomyces* spp. are well known to be responsible for significant economic losses because of their capacity to convert sugar into acetic acid (De Miniac 1989; Chatonnet et al. 1995; Délia-Dupuy et al. 1995; Gilis 1999). The increase in acetic acid concentration inhibits *Saccharomyces cerevisiae* growth and so diminishes the capacity for this yeast to produce ethanol (Gerós et al. 2000) and consequently brings about a decrease in the alcohol yield.

Because of these industrial problems, fermentation with *Brettanomyces* spp. has been extensively studied, particularly regarding (*i*) the influence of operating parameters, such as the pH of medium (Aguilar-Uscanga et al. 1999; Freer et al. 2003); (*ii*) the effect of oxygen (Wijsman et al. 1984; Gaunt et al. 1988; Ciani and Ferraro 1997; Aguilar-Uscanga et al. 2003); and (*iii*) the influence of ethanol concentration (Medawar et al. 2003). Nevertheless, there have been very few studies published on the influence of temperature on *Brettanomyces* yeast fermentation. Yet, temperature is one of the most important parameters in alcoholic fermentation, since it affects the kinetics of the process and can also modify yeast metabolism. For instance, it is reported that an increase in temperature from 15 to 35 °C causes a change in the cellular metabolism of *Saccharomyces cerevisiae* yeast, stimulating the formation of secondary products, such as glycerol, acetic acid, and succinic acid, among others (Lafon-Lafourcade 1983; Fleet and Heard 1993).

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The aim of this study was to increase the global knowledge of the *Brettanomyces* genus by providing information about the influence of temperature regarding 2 factors: metabolism and kinetics. The metabolic approach examines the effect of temperature on substrate distribution (glucose) between biomass and the main products of the metabolism (ethanol, acetic acid, carbon dioxide). The kinetic analysis evaluates the influence of temperature on metabolite production and growth rates and tries to correlate them. The effect of temperature on this yeast was studied through a set of aerated batch fermentations. Six different temperatures were tested: 15, 20, 25, 30, 32, and 35 °C. To minimize the complexity of this study, a synthetic minimum medium containing glucose as the only carbon source was used.

## Materials and methods

### Microorganism

The yeast strain used was isolated from a distillery plant contaminated by *Brettanomyces* yeast. It was identified as *Brettanomyces bruxellensis* by the Institute of Hygiene and Epidemiology Mycology of Brussels (IHEM), and registered under No. 6037.

### Culture media

*Brettanomyces bruxellensis* was stored at 4 °C on a solid medium whose composition was as follows (g/L): glucose, 20; agar, 20; and yeast extract, 10. For the preculture and for the experiments, the composition of the culture medium was as follows (g/L): glucose, 50; $KH_2PO_4$, 5; $(NH_4)_2SO_4$, 2; yeast extract, 1; and $MgSO_4 \cdot 7H_2O$, 0.4. The initial pH was adjusted to 4 using a 85% (*v*/*v*) orthophosphoric acid solution. The culture medium was then sterilized at 121 °C for 15 min.

#### *Preculture conditions*

The preculture was carried out in a 500 mL Erlenmeyer flask with 300 mL of liquid medium and was stirred magnetically at 250 r/min. After inoculation, each Erlenmeyer flask was incubated at 30 °C for 72 h. Two precultures were successively prepared to obtain well-activated yeast cells.

#### *Culture conditions*

The experiments were carried out in 2 L bioreactors (Setric Instruments, France) with a 1.6 L working volume. An aeration of 0.1 vvm was supplied, and the fermentors were mechanically agitated at 250 r/min. For the kind of reactor we used, these conditions correspond to a $K_La$ value of 6.4 /h. After sterilization, each fermentor was inoculated with the preculture suspension to obtain 3 million viable cells/mL. During fermentation, the pH of medium was not controlled. All experiments were carried out in duplicate.

### Analytical techniques

Six millilitre samples were withdrawn at regular time intervals. Biomass concentration was determined by 2 techniques: (*i*) a correlation between the optical density of yeast suspension at 620 nm and cell dry weight gave a weight concentration, and (*ii*) the use of a Thoma hemacytometer gave a concentration in number of cells. The percentage of viable cells was obtained using the methylene blue staining method, with an error of 8% (Lange et al. 1993). At the same time, 4 mL samples were centrifuged at 65 000*g* for 10 min. Then, the supernatant was frozen at –20 °C until its analysis. The glucose concentration was evaluated using an enzymatic analyzer device (Yellow Spring Instruments, Ohio, USA). Ethanol and acetic acid concentrations were determined with a high performance liquid chromatography (Thermo Separation Products, Spectra System Apparatus), using a BIORAD AMINEX HPX-87H column. The column was maintained at 40 °C, and the mobile phase was $H_2SO_4$ (0.005 mol/L) with a flow rate of 0.4 mL/min. The components were detected using a differential refractometer (TSP Refracto Monitor V). Specialized software allowed calculation of the detected peak surface. The experimental error did not exceed 3% for all the analyzed compounds.

### Analysis of exit gases

The oxygen uptake and carbon dioxide production were monitored on-line by means of a paramagnetic gaseous oxygen analyzer and an infrared $CO_2$ analyzer (SERVOMEX 4100 Gas Purity Analyzer) connected to the outlet of the bioreactor. The total amount of oxygen uptake and carbon dioxide produced was calculated by integration in time of the gases (Ramon-Portugal et al. 2004).

### Data treatment

The kinetic models (logistic and Luedeking-Piret models) were fitted to the experimental data, using a method to minimize the sum of squares of the error. To determine the sensibility of the calculated kinetic parameters, an analysis of variance (ANOVA) was carried out (Statgraphics Software).

## Results and discussion

The influence of temperature on *B. bruxellensis* fermentation was studied with batch experiments at 6 different temperatures ranging from 15 to 35 °C. The end of alcoholic fermentation was predefined to be when the residual glucose concentration was lower than 1 g/L.

### Influence of temperature on growth

The biomass evolutions are given in Fig. 1A for the 5 temperatures examined. In the temperature range of 15–32 °C, the final biomass concentration was very close to a maximum value of 5.6 g/L. However, when the yeast was incubated at 35 °C, the biomass production of *Brettanomyces* was strongly inhibited — the biomass diminished by 65% in comparison with the other tested temperatures and only attained 2 g/L. In terms of population (data not show), the same results were obtained — an average concentration of 375 million cells/mL for temperatures ranging between 15 and 32 °C and only 100 million cells/mL for 35 °C.

Kinetic profiles in Fig. 1A also show that an increase in the temperature had a positive effect on the growth rate and consequently on the end of the fermentation between 15 and 32 °C. In this range, the higher the temperature, the faster the production of biomass. For instance, the maximal biomass concentration was obtained after 220 h at 15 °C, whereas, the same maximal biomass production was obtained after 50 h at 32 °C. Nedwell (1999) reports that the

**Fig. 1.** Temperature effect on biomass concentration (A) and on percentage of viable cells (B) during fermentation.

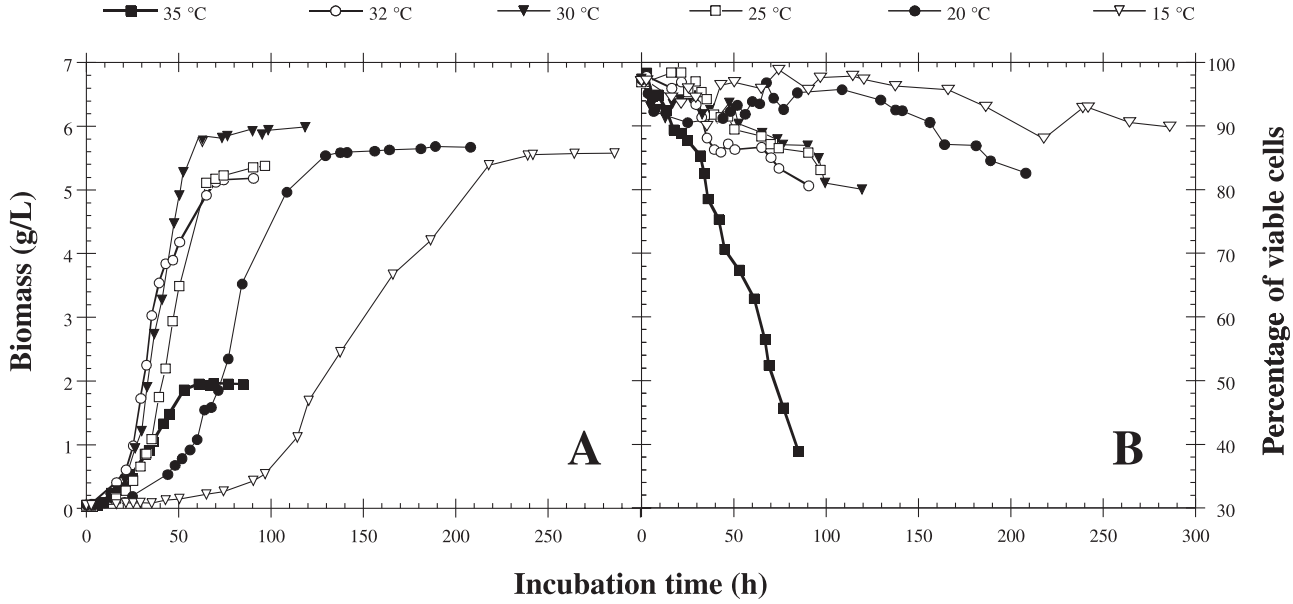
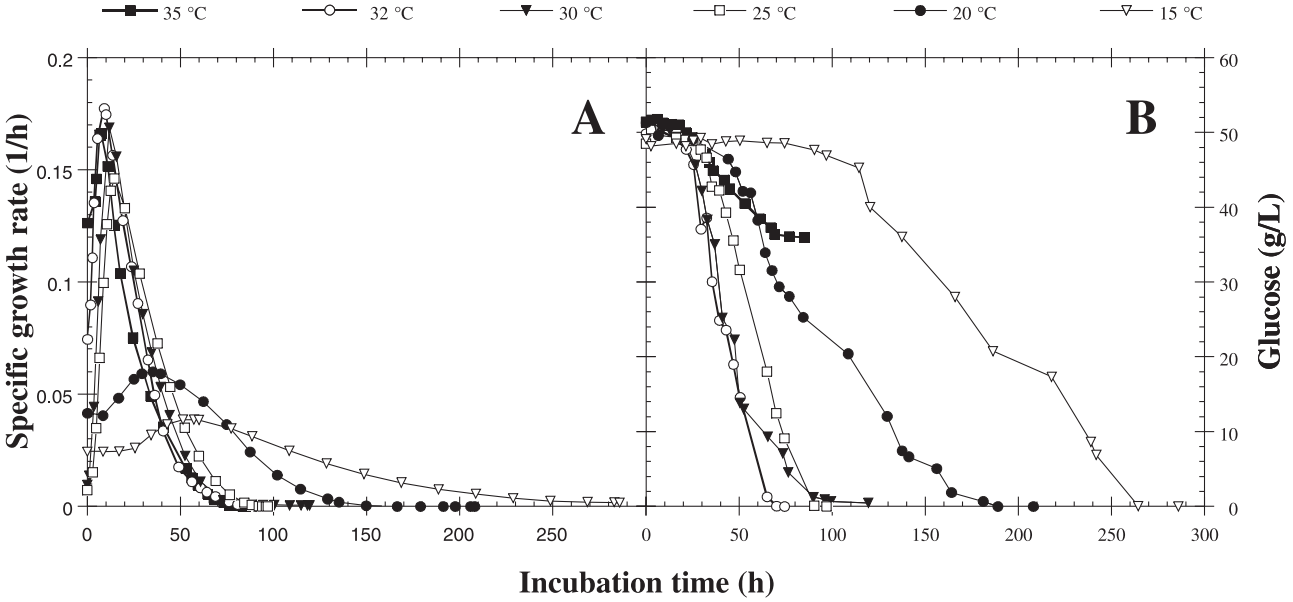



**Fig. 2.** Temperature influence on specific growth rate (A) and glucose consumption (B) during fermentation.




decrease in the microorganism growth rate when the temperature decreases is related to the reduction in membrane fluidity and is associated with the reduction in the affinity of the protein transport for substrates.

Figure 1B shows that the cell viability of *B. bruxellensis* was strongly influenced by the temperature. Fermentations between 20 and 32 °C remained at an average value of 86% viability until the end of the fermentation. At 15 °C, the highest viability percentage was obtained (not less than 90%) and remained constant during the entire fermentation. The kinetic profile at 35 °C shows a quick drop in viability to a value less than 40%. The high cell mortality may have induced a sluggish fermentation, and it could have produced a stuck fermentation with higher residual sugar contents. This behavior can be compared with results obtained by many authors for the influence of the fermentation temperature on different yeasts (Ough 1966; Nagodawithana et al. 1974; Casey et al. 1984; Torija et al. 2003). The latter studies showed that yeast cell viability decreases when the temperature increases. Cabeça-Silva et al. (1982) and Van Uden (1984) demonstrated that there is no intracellular accumulation of ethanol but that the target of temperature is the inner mitochondrial membrane. Lucero et al. (2000) showed equally that the temperature increase should damage the structure of the cellular membrane, decreasing its functionality.

Figure 2A shows the evolution in the specific growth rate during fermentation. The specific growth rate was greater than zero from the beginning of the fermentation, so there was no lag phase for all temperatures. Moreover, it was observed that the maximum specific growth rate value increased regularly with temperature up to a temperature threshold (32 °C), beyond which a fall took place, probably

**Table 1.** Comparison of activation energy ($E_a$) and $Q_{10}$ values for growth in different microorganisms.

| Microorganism | $E_a$ (kcal/mol) | $Q_{10}$* | Reference |
|---|---|---|---|
| *Brettanomyces bruxellensis* | 16.61 | 3.76 | This work |
| *Schizosaccharomyces pombe* | 26.2 | 3.33 | Taillandier 1990 |
| *Saccharomyces cerevisiae* | 12.29 | 2.05 | Saez 1986 |
| *Pachysolen tannophilus* | 13.59 | 1.86 | Sánchez et al. 2004 |

*For temperatures between 15 and 32 °C.

due to the progressive deactivation of the enzyme controlling growth or, at least, a decrease in its activity.

There are several models to evaluate the influence of temperature on microbial growth. In this work, we used the Arrhenius model and the $Q_{10}$ value. The Arrhenius equation, a well-known tool for the thermodynamic study of bioprocesses (Arni et al. 1999), describes the general dependence of the reaction rate constant on the temperature. It can be written for microbial processes as follows:

$$\mu = \mu_0 \, e^{\left(\frac{-E_a}{RT}\right)}$$

where $E_a$ = activation energy of cell growth (kJ/mol), $\mu_0$ = pre-exponential factor (/h), $R$ = ideal gas constant (kcal/mol), and $T$ = temperature (K).

From the maximum specific growth rate obtained for each incubation temperature (see Fig. 2A), we determined the parameters of the Arrhenius equation. We obtained a good correlation ($r^2 = 0.95$) for temperatures between 15 and 32 °C, with 16.61 kcal/mol for the activation energy and $1.56 \times 10^{11}$ /h for the pre-exponential factor.

The estimated $E_a$ for *B. bruxellensis* was comparable with the values found in the literature for other yeasts (Table 1). It appears that this strain was less sensitive to temperature than *Schizosaccharomyces pombe* ($E_a$ = 26 kcal/mol). On the other hand, the $E_a$ value indicates if the process is in a biological or a diffusion regime. With a biological regime (temperature directly influences kinetic parameters of growth) the $E_a$ is ≥12 kcal/mol, whereas, with a regime of diffusion (the system is affected by physical phenomena such as a limitation of the transfer of oxygen, mass transfer, coefficient of diffusion, etc) the $E_a$ is >12 kcal/mol (Sánchez et al. 2004; Serra et al. 2005). With this interpretation of $E_a$, we can conclude that we had a biological regime in our study.

Another tool to evaluate the influence of temperature on growth is the $Q_{10}$ value. This parameter represents the increase in the rate of the reaction when there is a 10 °C increase in temperature. The $Q_{10}$ value is calculated using the following equation (Urbano et al. 2005):

$$Q_{10} = \left(\frac{K_2}{K_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

where, $T_2$ is the higher temperature; $T_1$ is the lower temperature; $K_2$ and $K_1$ are the reaction rates at higher and lower temperature, respectively.

Also, like $E_a$, the $Q_{10}$ value can be used to determine if a process is physical ($Q_{10} \leq 1$) or biochemical ($Q_{10} \geq 2$). The $Q_{10}$ coefficient can also be a helpful tool to indicate the sensitivity of the reaction to a temperature increase within a defined range by measuring the changes in growth rates. In our study, for example, the $Q_{10}$ factor shows important changes related to the following temperature ranges: from 15 to 25 °C, the $Q_{10}$ value was 3.76, whereas, from 20 to 30 °C, this value decreased by 1 unit to $Q_{10}$ = 2.80. According to Larcher (2000), $Q_{10}$ values are higher at lower temperatures because in such conditions biochemical reaction is restricted by low enzymatic activity. At higher temperatures, the $Q_{10}$ values are lower because in such conditions a physical limitation appears, for instance, gaseous diffusion (decrease in $K_L a$ value).

Finally, we showed that $E_a$ and $Q_{10}$ are 2 tools that can be used to evaluate the influence of the temperature on growth. However, $E_a$ is more representative because it is a constant value on a range of temperature of growth unlike $Q_{10}$, which changes with the temperature range calculated.

## Metabolism

With regard to the metabolism of carbon by *B. bruxellensis*, Fig. 2B shows the residual glucose concentration in the culture medium. In all the experiments, glucose was completely consumed, except at 35 °C, where a stuck fermentation was observed and only 15 g/L of glucose was consumed. The low temperature had a negative effect on the glucose consumption rate. The lowest glucose consumption rate ($r_g$ = 0.18 g/(L·h)) was observed for fermentation at 15 °C, while this value was 3 times higher at 32 °C ($r_g$ = 0.55 g/(L·h)).

Figures 3A and 3B show the evolution of ethanol and acetic acid concentrations, the 2 main products of fermentation by *B. bruxellensis*. The ethanol concentration varied between 16 and 18 g/L for temperatures ranging from 15 to 32 °C, whereas, the acetic acid concentration varied from 3.4 to 4 g/L. At 35 °C, stuck fermentation was also observed for the products. Only 5.5 and 0.58 g/L of ethanol and acetic acid were produced, respectively, at this temperature, confirming the rapid death of the cells. For all temperatures studied, neither the ethanol nor the acetic acid caused an inhibition in glucose consumption or yeast growth during the fermentation. As for biomass production, high temperatures favoured ethanol and acetic acid production rates. This kinetic behaviour is consistent with the study of Freer (2002) on another strain of *Brettanomyces*.

A molar carbon balance was done to check the consistency of the experimental data and to verify if any secondary products of the fermentation were produced but not taken into account during the fermentation. The chemical formula of biomass ($CH_{1.54}O_{0.83}N_{0.107}$) proposed by Aguilar-Uscanga et al. (2003) for this yeast species was used for the calculation of carbon biomass. Measuring acetic acid, ethanol, and glucose concentrations made it possible to deter-

**Fig. 3.** Temperature effect on ethanol (A) and acetic acid (B) production.

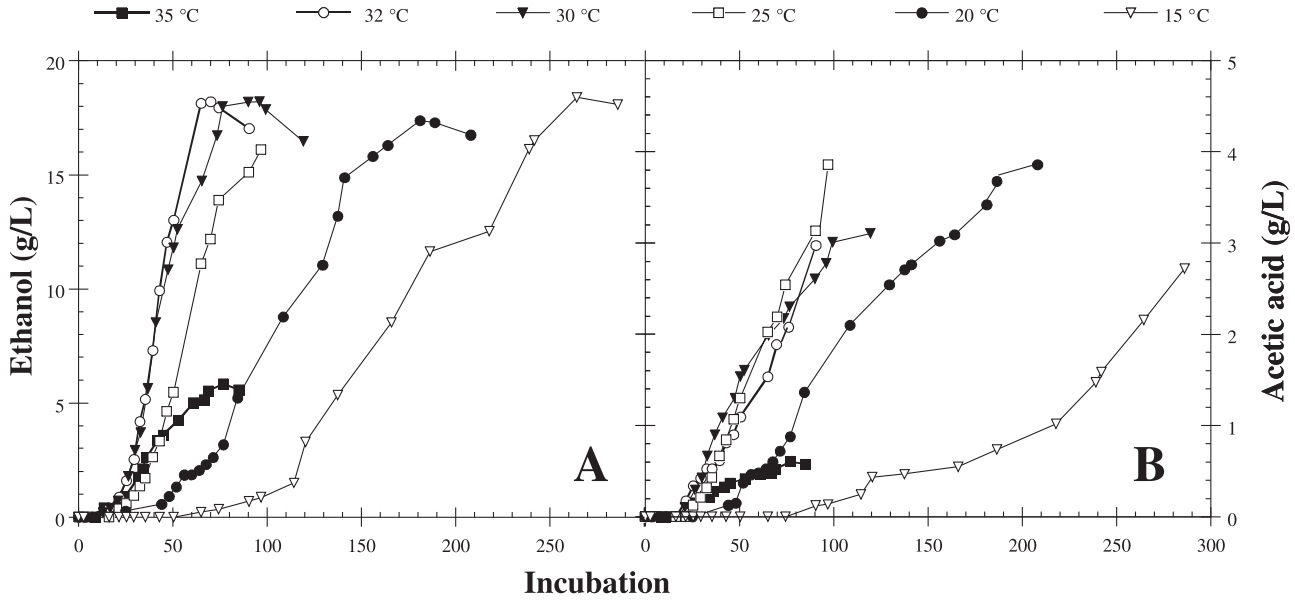
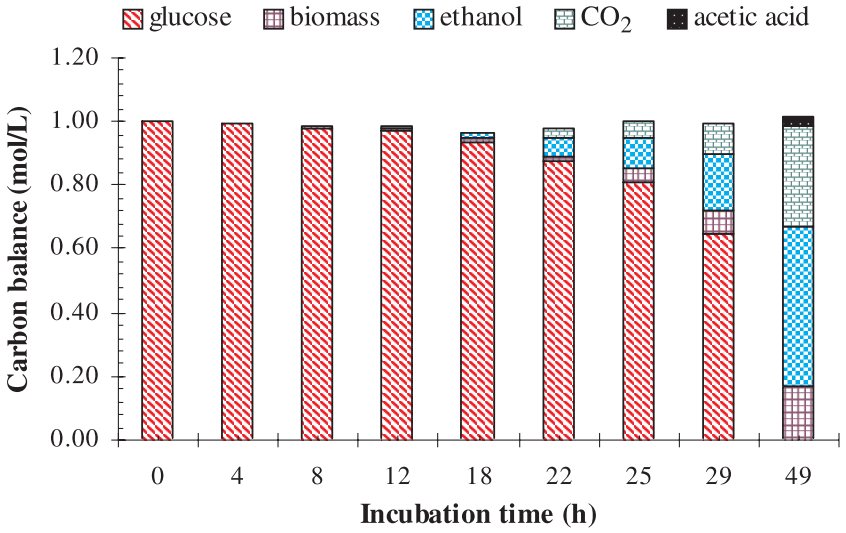



**Fig. 4.** Carbon balance at different times for a culture of *Brettanomyces bruxellensis* at 25 °C.




mine the carbon in the liquid substrate and products. The gas analysis of the fermentor outlet gave, after integration, the carbon atoms in the carbon dioxide produced. For the fermentation at 25 °C, global and instantaneous carbon balances were made. Figure 4 shows the evolution in the carbon balance between glucose and the different by-products of the fermentation.

The results show that the fermentation was well balanced, since the maximum error was less than 1.5% throughout fermentation. No secondary products were produced in any significant quantity. Thus, ethanol, acetic acid, biomass, and $CO_2$ were the products of the fermentation of *B. bruxellensis* from glucose.

For all temperatures between 15 and 32 °C, from the same glucose concentration (50 g/L = 1.67 mol of C), constant quantities of biomass (5.6 g/L = 0.198 mol of C) and by-products (1.472 mol of C) were obtained. Consequently, the distribution of glucose between biomass and by-products was not affected by temperature.

On the other hand, it is well known that under aerobic conditions, *Brettanomyces* yeast can use ethanol as substrate to produce acetic acid (Aguilar-Uscanga et al. 2003). In our experiments, we observed a slight decrease in ethanol at the end of the fermentation (Fig. 3A). We can suppose that the latter was converted into acetic acid. Nevertheless, parameters influencing the ethanol – acetic acid equilibrium are complex and not fully understood. Therefore, in this work, we have not studied this ethanol consumption phenomenon.

## Kinetic analysis

To make a kinetic analysis of these results, specific rates (production rates per gram of biomass) were calculated. To

**Fig. 5.** Representation of biomass concentration by the logistic model at 15 °C (A) and representation of ethanol and acetic acid concentration by the Luedeking–Piret Model (B).

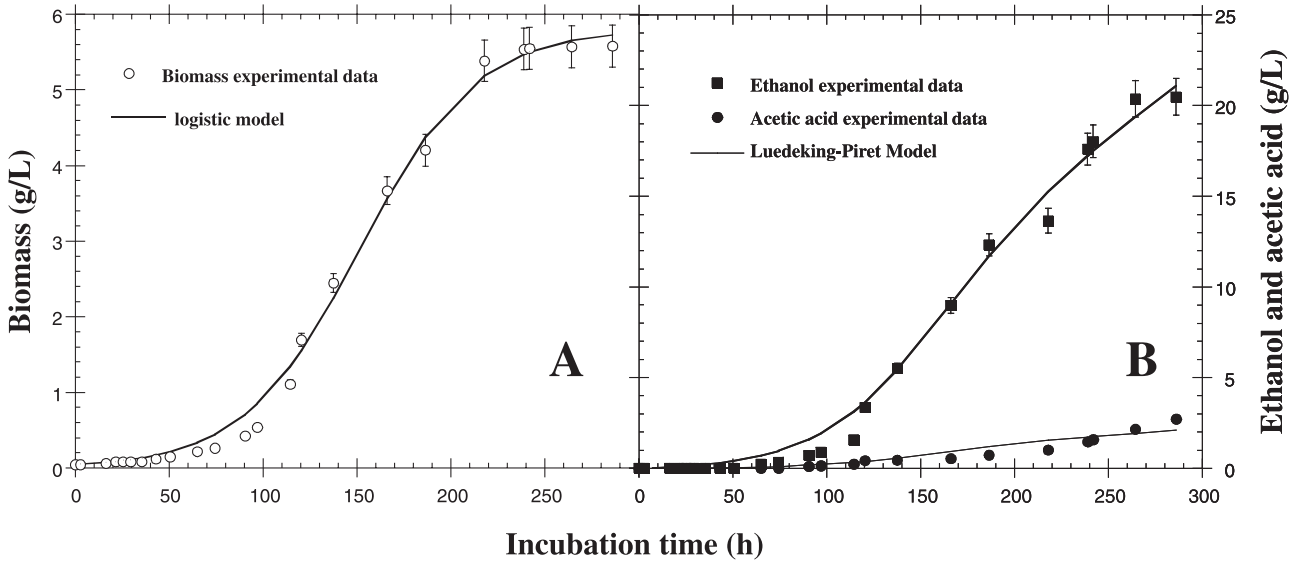
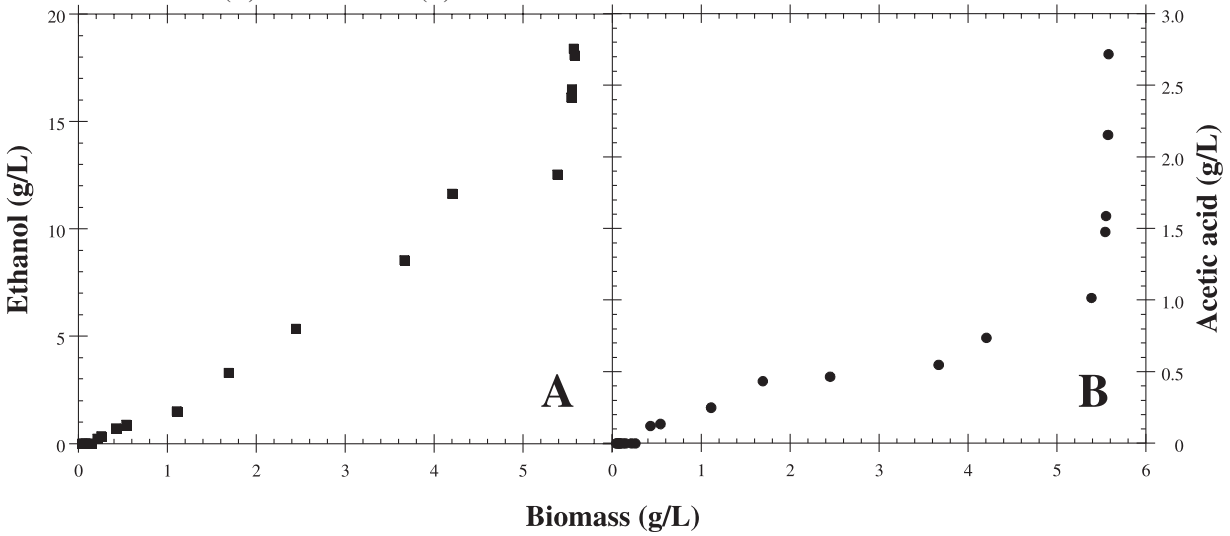



**Fig. 6.** Ethanol concentration (A) and acetic acid (B) as a function of biomass concentration at 15 °C.




avoid problems linked to the experimental errors in biomass concentration, we proposed to smooth the biomass concentration using a model and to calculate the specific production rate with the smoothed biomass values.

As shown in Fig. 1A, the profile of biomass production presented a sigmoid shape, and no lag phase was observed for all the temperatures evaluated. In this case, the logistic model should be used to calculate the specific growth rate (Verhulst 1845). The logistic model is widely used because it is well suited to describe the dynamics of the biomass in a batch culture.

The general form of the logistic equation is

$$[1] \qquad \frac{dX}{dt} = \mu_{max} X \left(1 - \frac{X}{X_{max}}\right)$$

The integration of eq. 1 gives

$$[2] \qquad X(t) = \frac{X_0 e^{\mu_{max} t}}{1 - \left(\frac{X_0}{X_{max}}\right)(1 - e^{\mu_{max} t})}$$

$X_{max}$ and $\mu_{max}$ are determined for each temperature by fitting the model with experimental data.

Figure 5A shows the good correlation between experimental points and modeled values for biomass concentrations (example at 15 °C).

The results shown in Figs. 1 and 3 imply a correlation between growth rates and product rates for all temperatures. Figures 6A and 6B confirm that there is a linear relationship between biomass and ethanol and between biomass and acetic acid for low concentrations of biomass, but at the end of the fermentation (when the biomass concentration was constant), ethanol and acetic acid production appear to not be linked to cellular growth.

**Table 2.** $\alpha$ and $\beta$ values calculated with the Luedeking–Piret model at different temperatures and correlation coefficients ($R$).

| | Ethanol | | | Acetic acid | | |
|---|---|---|---|---|---|---|
| Temperature (°C) | $\alpha_{eth}$ | $\beta_{eth}$ | $R^2$ | $\alpha_{aa}$ | $\beta_{aa}$ | $R^2$ |
| 15 | 1.967%±18% | 0.013%±24% | 0.99 | 0.213%±20% | 0.001%±17% | 0.96 |
| 20 | 1.967%±18% | 0.012%±18% | 0.98 | 0.213%±20% | 0.004%±10% | 0.99 |
| 25 | 1.967%±18% | 0.022%±21% | 0.98 | 0.213%±20% | 0.009%±17% | 0.99 |
| 30 | 1.967%±18% | 0.042%±31% | 0.98 | 0.213%±20% | 0.006%±14% | 0.99 |
| 32 | 1.967%±18% | 0.047%±35% | 0.98 | 0.213%±20% | 0.005%±16% | 0.97 |
| 35 | 1.967%±18% | 0.031%±12% | 1.00 | 0.213%±20% | 0.002%±7% | 1.00 |

**Note:** eth, ethanol; aa, acetic acid.

The Luedeking–Piret model (Luedeking and Piret 1959) was used to test mathematically these observations.

The Luedeking–Piret model is written as follows:

$$[3] \qquad \frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X$$

where $P$ = product concentration (ethanol or acetic acid), $X$ = biomass concentration, $\alpha$ = constant associated with growth, $\beta$ = constant associated with the population. Then, eq. 2 substituted into eq. 3 and after integration gives

$$[4] \qquad P(t) = P_0 + \alpha K_1 + \beta K_2$$

where

$$[5] \qquad K_1 = X - X_0$$

and

$$[6] \qquad K_2 = \frac{X_{max}}{\mu_{max}} \ln \left[ 1 - \frac{X_0}{X_{max}} (1 - e^{\mu_{max} t}) \right]$$

The $\alpha$ and $\beta$ parameters were determined for each experiment and for each product by fitting the model to the experimental data. A correlation coefficient higher than 0.96 was obtained for all the experiments, which suggests that the data fitted a straight line (Table 2). Thus, the Luedeking–Piret model well represents the ethanol and acetic acid concentrations for a temperature of 15 °C (Fig. 5B). Both the logistic model and the Luedeking–Piret model can be applied to the present fermentation system with a good degree of confidence.

The ANOVA analysis shows that $\alpha$ and $\beta$ were significant with a probability greater than 95%. Confidence limits calculated for these parameters were 18% and 20% for $\alpha_{eth}$ and $\alpha_{aa}$, respectively, between 18% and 35% for $\beta_{eth}$, and between 7% and 17% for $\beta_{aa}$.

$\alpha$ and $\beta$ parameters were not equal to zero and the $\alpha K_1$ and $\beta K_2$ terms had a nonnegligible influence on the total amount of ethanol and acetic acid produced, meaning that the each product rate was only partially proportional to the growth rate. The results from the application of the Luedeking–Piret model were constant $\alpha_{eth}$ and $\alpha_{aa}$ values and variable $\beta_{eth}$ and $\beta_{aa}$ values regardless of temperature. In our analysis of *Brettanomyces* metabolism, this result proved very interesting.

Constant $\alpha_{eth}$ and $\alpha_{aa}$ values meant that for 1 g of formed biomass, 1.97 g ($\alpha_{eth}$) of ethanol and 0.21 g ($\alpha_{aa}$) of acetic acid were produced regardless of temperature. So, the same reaction system with only one reaction rate is involved in the production of biomass, ethanol, and acetic acid. Temperature influenced the kinetic parameters of this reaction but not the metabolic parameters involved in distributing the substrate between the biomass and the main metabolic products.

Moreover, ethanol and acetic acid can be produced by cells in stationary phase. $\beta_{eth}$ and $\beta_{aa}$ values signified that even if a cell was in stationary phase, it was able to ferment glucose to produce ethanol and acetic acid. For a given temperature, the rate of production was linked to the biomass quantity. So, the concentration of ethanol and acetic acid was the sum of the quantity produced by cells in growth and by cells in stationary phase. The $\beta$ evolution with temperature was different for ethanol and acetic acid: the optimal $\beta_{eth}$ value was obtained at 32 °C, whereas, optimal $\beta_{aa}$ value was obtained at 25 °C. This observation proved that 2 different enzymatic systems are involved in the production of ethanol and acetic acid, and each system had its optimal temperature.

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