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CALORIMETRIC CHARACTERIZATION OF DIFFERENT YEAST STRAINS IN DOUGHS

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Abstract

In this work microbial growth and metabolism of *Saccharomyces cerevisiae* from three different strains in a bread dough system were investigated by means of SETARAM C80D twin calorimeter in isothermal conditions at various temperatures. The overall calorimetric signal accounts for two main contributions, namely oxidative and anaerobic degradation of substrate.

Parallel measurements of the biomass growth $N(t)$, the substrate (maltose) consumption, fermentation gases (CO_2) production, dough volume increase, were also performed. Differences were observed between the three yeasts both in the metabolic energetics and growth and metabolism kinetics.

Keywords: calorimetry, dough, microbial growth

Introduction

Parameterization of microbial growth and metabolism is of main interest in predictive bacteriology since food biotechnology often requires use of such parameters to model the production of specific foods and/or food ingredients, like fermented beverages, cheeses and other dairy products and doughs. In these cases the possibility of a kinetic description of the microbial action is expedient to improve process conditions and related yield [1].

Yeasts, and particulary *Saccharomyces cerevisiae* species, are largely employed in food industry in biotransformations of specific substrates (musts, doughs) through anaerobic metabolism (alcoholic fermentation with CO_2 release) and/or aerobic biomass production (pressed baking) [2, 3].

The close relationship between metabolic activity and heat release allows a successful use of calorimetry to monitor this biological process and recognize different yeast performances [4]. Although many studies are devoted to characterize the growth and metabolic activity of yeasts in model systems (broths, artificial solid substrates, etc.), investigations of real yeast-in solid substrate systems have so far received small attention.

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In this work microbial growth and metabolism of *Saccharomyces cerevisiae* in a bread dough system were investigated in different isothermal conditions by means of calorimetric and classic microbiological methods (plate count, substrate consumption, CO_2 release). Furthermore, since the performance of a yeast is largely determined by its genetic constitution, the metabolic behaviours of three different *Saccharomyces cerevisiae* strains were investigated.

The technological role of yeast in a dough (mixed water – flour viscous system) is the promotion of the fine and homogeneous formation of CO_2 bubbles, that drastically contribute to the expanded structure of the baked product. The quality of final products, mainly for their mechanical properties, are largely related to such mechanism.

Materials and methods

Strains and classical tests

Three strains of *Saccharomyces cerevisiae* (A, B, C) were isolated from three different commercial pressed baking, according to techniques of the classical microbiology, by use of malt agar [5].

The isolated strains were maintained at 4°C and transferred monthly. A fresh culture, prepared by suspending isolated strains in distilled water, was inoculated in several 20 g dough samples, obtained by mixing (1:2 w/w) distilled water and patent flour (Molini M.V.M, Erba, Como, Italy). The yeast was added to attain a final concentration of about 10^3 CFU/g (CFU = Colony Forming Unit).

These samples were used for culture tests lasting for 100 h in isothermal conditions (25, 30 and 35°C) at controlled humidity: the microbial population was determined at various times by sterile sampling and incubating at the given temperature in the same medium used for isolation. The same sample was concurrently used for quantitative determination of maltose (Boehringer enzymatic kit) to evaluate the fermentation progress. Plate counts and fermentation indices reported for each sample were the average of three replicas.

The considered strains differ from one another because of the affinity for various saccharides and/or oligosaccharides present in the flour. On the basis of assimilation tests [6], strain A showed a faster maltose consumption and a narrower assimilation spectrum of other mono- and oligosaccharides. It is therefore more suitable for leavening bread doughs.

Dough volume expansion

The volume increase was evaluated in a separate test by image-analysis. Several 10 g dough samples, prepared and inoculated in the same way as above, were settled between sterile glass plates kept at 0.2 mm apart, in order to allow only a

radial expansion. The glass plates were maintained in the same isothermal conditions as above. Images of the plates were acquired at different times by means of a desk-scanner (Hewlett Packard, DeskJet II, gray level, 100 dpi resolution). Every image was processed by means of Image-Pro Plus software (Media Cybernetics, Maryland, USA) to measure the surface area of the expanding dough, which is proportional to the actual dough volume. Data were reported as V/V_0, where V_0 is related to the surface area at zero time.

Calorimetric measurements

A SETARAM C80D differential flow calorimeter equipped with cylindrical stainless steel cells (10 cm length, 4.5 mL available volume, with a small hole to allow gas exchange) was used for calorimetric investigations. 3 g inoculed dough sample was loaded into the measurement cell. To avoid superposition between signal stabilization (2 h were typically required to reattain the base-line level) and the start of significant metabolic activity (10 h), the initial yeast population was kept adequately low (10^3 CFU/g). The reference cell contained 3 mL of sterile water. The trace showed a broad exothermic (upward) peak followed by a wide downward wave. The original base-line level was eventually recovered after 70–80 h.

The calorimetric trace, recorded in ASCII format, was smoothed via FFT and scaled from the base-line by means of TableCurve software (Jandel, Germany) and an Ifestos (a dedicated software implemented in our laboratory) calculation routine (Fig. 1). All the measurements were repeated three times. In spite of the variability of dough preparation, the reproducibility of the calorimetric signals was satisfactory (ΔH error <5%).

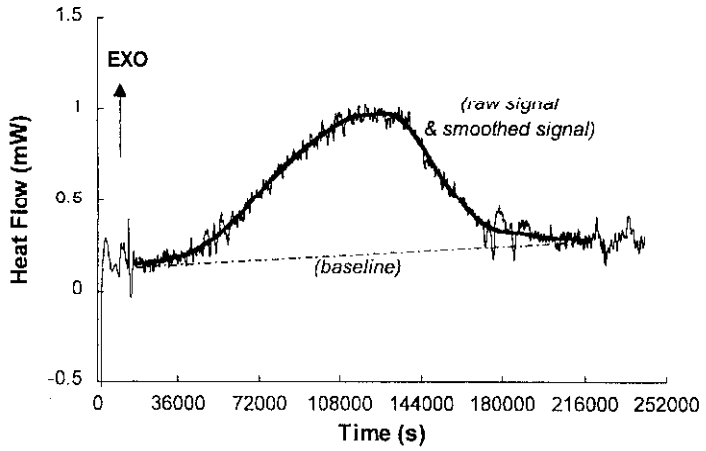

Fig. 1 Yeast strain A, 30°C: raw DSC trace, smoothing and base-line correction

Results and discussion

Figure 2 reports the cell population trend of the three yeast strains at 30°C. Strains A and B showed an increase of the microbial population, N, from the starting value N_o up to a plateau, N_{max}, followed by a decline toward a steady level below N_o, within a 60 h time span. On the contrary, this phase is absent in strain C.

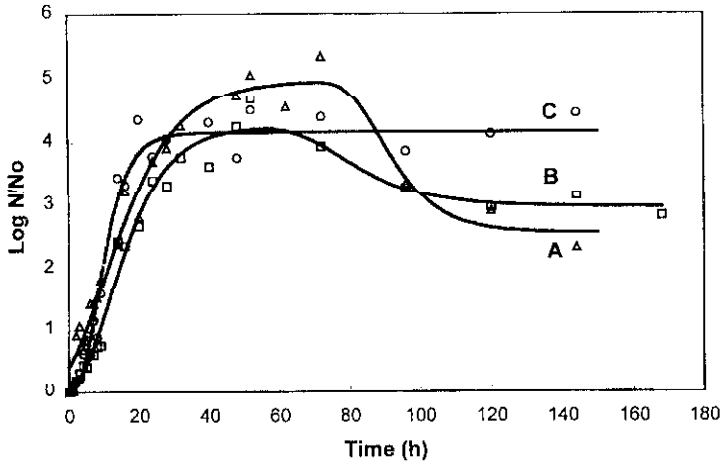

Fig. 2 Trend of the microbial population (Log_{10} units) of the three yeast strains according to Eq. (2). The relevant parameters of the fit are reported in Table 1

A number of fitting functions have been so far exploited to describe the increase of a microbial population. Among these, the modified Gompertz function, $G(t)$, was often found more adequate than others to work out the experimental results (7–9):

$$G(t) = \ln\frac{N}{N_o} = a\exp[-\exp(b - ct)] \quad (1)$$

The conventional parameters, namely, microbial population, N, lag-phase, $\lambda = ((b-1)/c)$, maximum growth rate, $\mu = ac/e$ (e being the base of natural logarithms) and plateau level, $\ln(N_{max}/N_o) = a$ are accordingly accounted for.

Equation (1) was modified in the present work by including a descendant sigmoidal function to describe the microbial death. For the sake of simplicity a Gompertz function with a negative derivative, G_d, was added to the traditional function.

$$G_{gr} - d(t) = \ln\frac{N}{N_o} = G_{gr} - G_d = a\exp[-\exp(b - ct)] - d\exp[-\exp(e - ft)] \quad (2)$$

The comparison of the Gompertz parameters (Table 1) shows that in every case the lag time is rather short (for strain A practically undetectable) and the population maximum attains the same order of magnitude. Strains C seem to grow faster than strain A and B, his μ_{gr} being significantly larger than for strain A and B. The faster growing strain C however attains a lower N_{max} and seems to survive for a longer time.

Table 1 Relevant parameters of microbial growth for the different yeast strains (T=30°C)

	Strain A	Strain B	Strain C
λ=lag phase/h	0	3.2	3.3
$t_{\mu gr}$=time of max growth rate/h	10.1	12.0	8.4
μ_{gr}=max growth rate/CFU g $^{-1}$h^{-1}	1452	1506	2074
t_c=start of decay/h	79.4	64.1	–
N_{max}=max number of microorganism/CFU g^{-1}	$9.3 \cdot 10^7$	$1.75 \cdot 10^7$	$1.68 \cdot 10^7$

The related DSC traces are reported in Fig. 3.

According to previous investigations dealing with other microbial species [10, 11], the calorimetric traces should account for thermal effects related to both growth and metabolism, the amplitude of the peak signal being usually related to the maximum growth rate. Following this interpretation strain A would grow and decline in a faster way than the other two strains, and strain C would have a delayed and slower evolution. However this is not the present case since growth data (Table 1) support the opposite interpretation.

To rule out possible effects due to the different environment (oxygen availability) of plate count determinations and calorimetric runs, specific culture tests

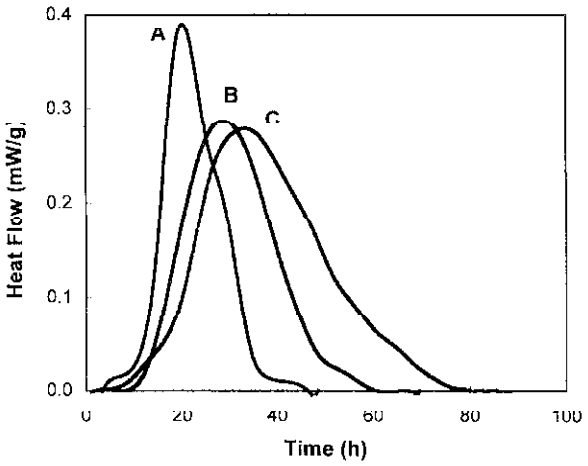

Fig. 3 Comparison of DSC traces obtained from the three yeast strains at 30°C

were performed on dough samples incubated in the calorimetric cell. This investigation revealed that after 10 h (i.e. at the onset of calorimetric peak) the yeast cell population had already attained almost its maximum value ($0.7 \cdot 10^7$ CFU/g). Therefore the microbial growth escaped any calorimetric detection. Accordingly, the calorimetric signal had to be matched only with the metabolic activity of the yeast. This conclusion holds for all the strains considered.

Although some overlap seems exist between volume expansion and maltose consumption, as shown in Fig. 4, the rates of population growth, dough volume expansion, substrate consumption do not allow recognition of direct correlations with heat flow.

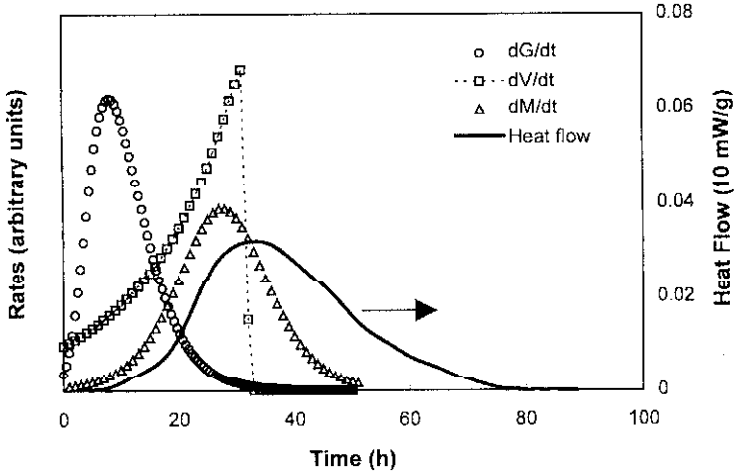

Fig. 4 Yeast strain C, 30°C: comparison of DSC trace and rates of microbial growth dG/dt, maltose consumption dM/dt, and dough volume expansion dV/dt

Table 2 shows some phenomenal parameters dealing with metabolic activity and calorimetric trace. These parameters were drawn from expressions used to fit experimental data of volume expansion (asymmetric sigmoid), maltose consumption (symmetric sigmoid), and heat flow (sum of two gaussian functions).

No correlation were found between overall maltose consumption and volume expansion: part of the substrate indeed remains as a residue in the case of strain B and C cultures which sustain larger volume increase, while strain A rapidly and exhaustively consumes all the available substrate with a poorer dough expansion. Analogous lake of correlation was found between overall enthalpy measured and maltose consumption. Reliable matching seems instead possible between heat flow and volume expansion (Fig. 5) and between the time of attainment of maximum peak signal and the time of maximum rate of maltose decrease. Some suggestion for the interpretation of the overall picture of the experimental evidence comes from the apparent correlation between maximum rate of maltose consumption and maximum population level: it seems indeed that the

Table 2 Relevant parameters of volume expansion, maltose consumption and thermal effects for the different yeast strains (T=30°C)

	Strain A	Strain B	Strain C
Volume expansion			
Time of max rate/h	18.7	19.1	31.7
Max rate $[d(V/V_0)/dt]/h^{-1}$	0.081	0.096	0.086
Max V/V_0	1.97	2.11	2.32
Maltose consumption			
Time of max rate/h	18.0	25.0	27.1
Max rate/g% h^{-1}	0.215	0.045	0.038
Total consumed maltose/g%	2.58	1.41	1.00
Heat flow			
ΔH/J g^{-1}	21.70	23.39	31.06
Time of max heat flow/h	20.0	28.5	33.1
Time range/h	8–40	9–60	6–77

larger N_{max}, attained in the early phases of the experiment, the larger the rate of maltose uptake (Fig. 5).

A simple check however showed that the actual average number of alive cells in the time span where the calorimetric signal was detectable, was approximately the same for all the strains considered, namely about $1 \cdot 10^7$ CFU/g of dough. This implies that differences in overall thermal effect cannot be ascribed to different microbial populations. It seemed instead reasonable to take into consideration a different metabolic balance of maltose for the three strains, with different proportions between oxidative and fermentative pathways.

The literature provides the relevant enthalpy change for glucose, namely 11.1 kJ g^{-1} and 550 J g^{-1} for oxidative and fermentative reaction, respectively [12]. Since these values differ by more than one order of magnitude, they can be tentatively applied to other sugars and related oligosaccharides, like maltose.

A rough estimation of the proportion between the extent of oxidative and fermentative reactions can be approached by assuming that the observed overall enthalpy change can be split into two contributions, namely,

$$\Delta_{tot}H = m_{maltose}[X\Delta_{ox}H + (1-X)\Delta_{ferm}H] \quad (3)$$

where $\Delta_{tot}H$, $\Delta_{ox}H$, and $\Delta_{ferm}H$ stand for experimental thermal effect, enthalpy of maltose oxidation (to CO_2 and H_2O) and maltose fermentation, respectively;

$m_{maltose}$, and X stand for maltose consumption per gram of dough and oxidation-to-fermentation proportion.

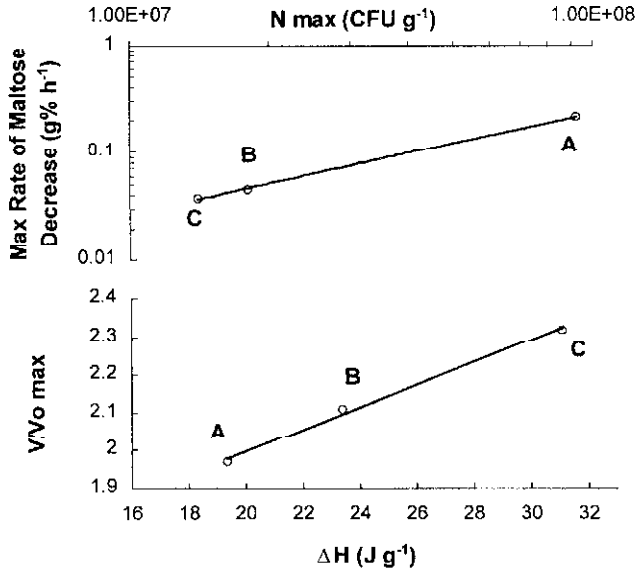

Fig. 5 Apparent correlation between maltose decrease maximum rate and maximum cell population (upper). Apparent correlation between maximum volume expansion and heat flow (lower)

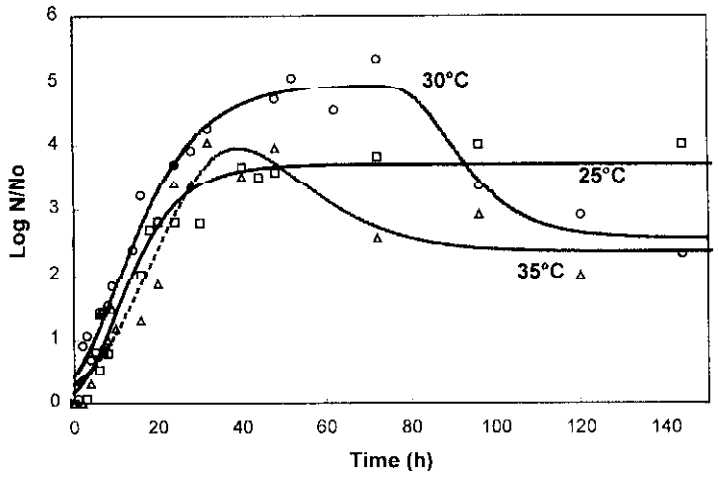

Fig. 6 Trend of the microbial population (Log_{10} units) of yeast strain A at three temperatures according to Eq. (2). The relevant parameters of the fit are reported in Table 3

Accordingly, the calculated X values were: 0.02, 0.10, and 0.26 for strain A, B, and C, respectively. Although the above estimation did not take into account the possibility that yeast could consume other substrates available in the dough, the X values seemed consistent with the earlier and faster growth of strain A which would exhaust the available oxygen (growth of *S. cerevisiae* mainly occurs in aerobic conditions) and therefore sustain an anareobic metabolism of the maltose uptake.

Such a behaviour should be considered more favourable for technological applications, since it implies a thorough fermentative consumption of the oligosaccharide substrate, which implies larger amounts of aroma compounds, and an earlier formation of the dough texture.

Strain A was therefore considered to extend the investigation to the effect of temperature. It is well known that within the temperature range where *S. cerevisiae* can survive and grow up, namely 15–40°C, all process rates are largest at the optimal temperature of 30°C, and tend to increase with temperature: Figs 6 and 7 report this effect for microbial growth, volume expansion and calorimetric signal. The related parameters (Gompertz, volume expansion and heat flow) are summarized in Table 3. Results obtained at 30°C can be summarized as follows: the lag phase is almost neglectable, μ_{gr} attains the largest value in the shortest time, the largest cell population (one order of magnitude more than at 25 and

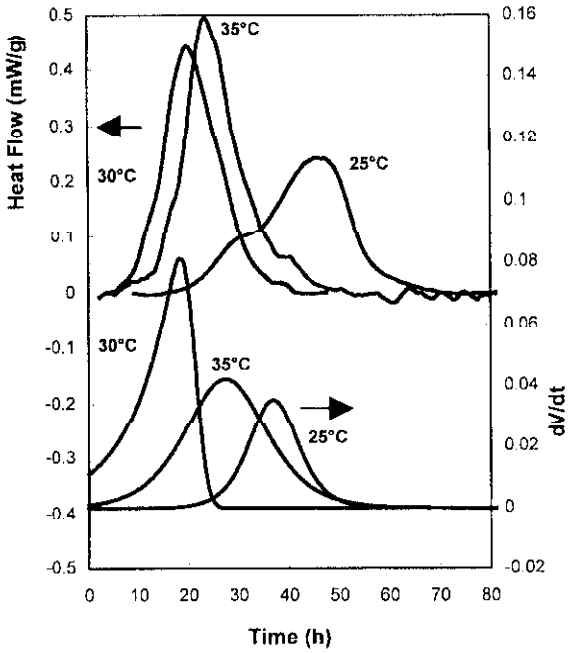

Fig. 7 Yeast strain A: comparison between heat flow and volume expansion rate observed at three temperatures

35°C) is attained, the maximum rate of volume expansion is the highest and the related time the shortest; the onset of the calorimetric signal is the earliest.

Table 3 Relevant parameters of microbial growth, volume expansion and thermal effects at different temperatures (Strain A)

	25°C	30°C	35°C
Microbial growth			
λ=lag phase/h	1.13	–	2.85
$t_{\mu gr}$=time of max growth rate/h	9.7	10.1	20.9
μ_{gr}=max growth rate/CFU $g^{-1}h^{-1}$	1444	1452	1382
t_c=start of decay/h	–	79.4	32.1
N_{max}=max number of microorganism/CFU g^{-1}	$5.2 \cdot 10^6$	$9.3 \cdot 10^7$	$9.0 \cdot 10^6$
Volume expansion			
Time of max rate/h	36.9	19.1	27.4
Max rate $[d(V/V_0)/dt]/h^{-1}$	0.035	0.081	0.042
Max V/V_0	1.45	1.96	2.00
Heat flow			
ΔH/J g^{-1}	18.42	21.70	24.25
ΔH_1/J g^{-1} (first gaussian area)	4.05	2.41	10.60
ΔH_2/J g^{-1} (second gaussian area)	14.37	19.29	13.65
Time of max heat flow/h	45.4	20.0	23.5
Time range/h	19–66	8–40	10–46

The thermal effects (Fig. 7) observed at the three temperatures deserve some specific comment: at 30 and 35°C the results obtained were 22 and 24 J g^{-1}, respectively, while a smaller effect was found at 25°C (18 J g^{-1}); the actual average number of alive cells in the time span where the calorimetric signal was detected (Table 3) was about $1 \cdot 10^7$/g of dough at 30°C, while it was $4 \cdot 10^6$ at 25 and 35°C. Once again the faster microbial growth at 25°C with respect to that at 35°C (9.7 *vs.* 20.9 h) can justify a smaller oxygen content available for the subsequent metabolism and therefore a smaller contribution to the overall thermal effect. When Eq. (3) is applied to these data, the calculated proportion between oxidative and fermentation reaction is 1.5, 2.7, and 6.3% for 25, 30 and 35°C, respectively. The signal in the trace obtained at 25°C shows a neat shoulder that can be reliably separated by deconvolution (Fig. 8). The area underlying the related two-gaussian fit indeed accounts for a 1.4% proportion of the oxidative reaction and therefore supports the above conclusions.

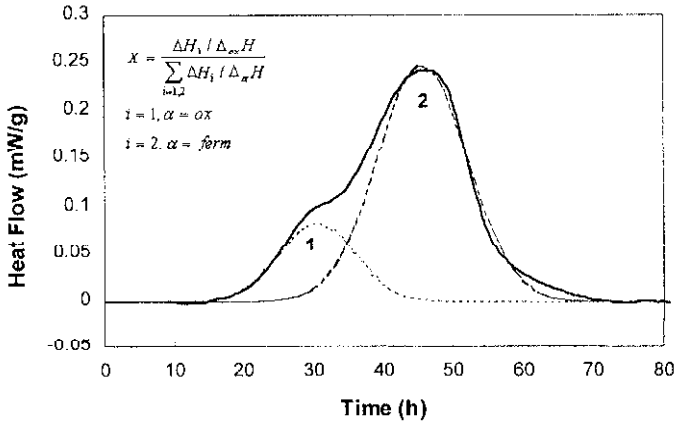

Fig. 8 Deconvolution of the DSC signal obtained from strain A added dough incubated at 25°C. The two gaussian contributions were referred to as oxidative (ox) and fermentative (ferm) process. For the meaning of X, see text

Conclusions

This work shows that calorimetry can be reliable applied to the study of microbial metabolism also in very complex media, like bread doughs, which are of specific technological interest. The previous knowledge about metabolism of *S. cerevisiae* in simple culture media have been substantially confirmed, mainly for what concerns the optimal temperature and choice of the strain. Oxidative degradation and anaerobic fermentation of the substrate can be recognized from the shape of the calorimetric signal and matched with data from plate count investigations and consumption of the feeding substrate (maltose).

Strain A, as expected from its specific affinity for maltose, showed better performances, namely, a larger growth and a larger proportion of anaerobic *vs.* aerobic maltose degradation. The best technological performance of this strain observed in the 25–30°C range can be explained with an early oxygen consumption to sustain the microbial growth within the dough and therefore a larger proportion of the anaerobic degradation of the substrate which is desirable in view of its flavour side-products.

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