Zymomonas mobilis: biomass production and use as a dough leavening agent

Alida Musatti · Manuela Rollini · Cecilia Sambusiti · Matilde Manzoni

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A. Musatti · M. Rollini · M. Manzoni
DeFENS, Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy

C. Sambusiti
DICA, Department of Civil and Environmental Engineering, Environmental Section, Politecnico di Milano, Piazza L. da Vinci, 32, 20133 Milan, Italy

M. Rollini (✉)
Via Mangiagalli 25, 20133 Milan, Italy
e-mail: manuela.rollini@unimi.it

Introduction

In Western countries, alcoholic beverages such as beer, wine and cider are generally produced by fermentation with yeasts, the most common among them being *Saccharomyces cerevisiae*. In many tropical areas of America, Asia and Afri-ca, other types of alcoholic beverages are very popular and widely produced: these consist of plant saps undergoing fer-mentation processes by mixed cultures containing bacteria belonging to the genus *Zymomonas*. In Europe, bacteria of this genus can be found occasionally as contaminants of beer, cider and perry (Swings and De Ley 1977; Sahm et al. 2006; Alcantara-Hernandez et al. 2010).

Zymomonas mobilis is an asporigenous Gram-negative bacterium, able to consume only glucose, fructose and sucrose, via the Entner-Doudoroff (ED) metabolic pathway, giving an equimolar mixture of ethanol and CO_2. *Zymomonas mobilis* produces ethanol faster than *Saccharomyces cerevisiae*, the most popular ethanol producer, and also has both a high sugar tolerance (up to 400 g/L) and ethanol resistance (up to 12 %) (Sprenger 1996). Many studies have investigated the advantages of the use of *Z. mobilis* in ethanol production instead of *S. cerevisiae*. This research line is lively, and the depletion of fossil fuel reserves is pushing research towards alternative energy sources (Panesar et al. 2006; Schuster and Chinn 2013). Regarding ethanol production, several studies have been trying to widen the range of fermentable substrates and to reduce the formation of by-products through genetic manipulation of *Z. mobilis* (Lawford and Rousseau 2003; So

et al. 2014). Moreover the production of organic acids (Malvessi et al. 2013), sorbitol (Cazetta et al. 2005; De Barros and Celligoi 2006) or levan (De Oliveira et al. 2007), considered by-products in the ethanol production process, have been a focus of great attention.

On the contrary, the ability of *Z. mobilis* to produce CO_2 has rarely been exploited. Like with *S. cerevisiae*, which is used in bakery products, the gas evolved can be used to leaven a dough. Perhaps the fact that *Z. mobilis* has a low molar growth yield—among the lowest known for bacteria—may have discouraged any research into its biomass production and application. *Z. mobilis* metabolism leads to a lower energy yield compared to *S. cerevisiae*: only a small percentage (< 3 %) of sugar consumed by *Z. mobilis* ends up in biomass (Sahm et al. 2006). To date, the only published study relating to investigation of the leavening ability of five *Z. mobilis* strains is insufficient to conclude if these bacteria could be used in bread making (Oda and Tonomura 1994).

In recent years in Western countries the incidence of yeast intolerance, due to an adverse immune response towards mannans, has been increasing (Annese et al. 2004; Brunner et al. 2006); *S. cerevisiae* has also been related to fungemia in immunosuppressed and critically ill patients (Munõz et al. 2005). Therefore, these health issues may justify deeper investigation into the use of *Z. mobilis* in dough production.

In the present study, we investigated the use of *Z. mobilis* as alternative to the commercial yeast *S. cerevisiae*. This study is the first step in research aimed at optimizing *Z. mobilis* biomass growth yield (despite the fact that yields are much lower than with *S. cerevisiae*): we evaluated *Z. mobilis* growth performance in media with glucose and fructose as energy source, avoiding the ingredient yeast extract. The best condition was then up-scaled to a pilot plant fermenter, and the biomass obtained was employed in dough-model systems to evaluate *Z. mobilis* leavening performance compared with *S. cerevisiae*.

The potential baked product will be targeted at people having adverse responses to the ingestion of bakery food leavened with *S. cerevisiae*.

Materials and methods

Microorganism

Zymomonas mobilis DSMZ 424 (DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) and DSMZ 3580 were employed in this research. These strains were chosen on the basis of their fermentative performance, as reported by Oda and Tonomura (1994). A sample of baker's yeast strain in compressed form (*Saccharomyces cerevisiae*, AB Mauri, Italy) was employed as reference. Yeast was stored at 4 °C and used in the 1st week after purchase.

Culture conditions

Strains were maintained weekly in DSM liquid medium of the following composition (g/L): bacto-peptone (Costantino Spa, Turin, Italy) 10, yeast extract (Costantino Spa) 10, glucose 20, pH 6.8, sterilization 118 °C for 20 min. Cultures were inoculated (10 % v/v) with a pre-culture in the same medium, incubated at 30 °C for 24 h in stationary conditions and subsequently stored at 5 °C. Cultures grown in DSM medium were used as inoculum of the following biomass production trials.

Zymomonas mobilis growth in flasks

Biomass was grown in 300 mL and 1 L flasks containing 200 and 600 mL, respectively, of the following IC medium (g /L): bacto-peptone 10, casein enzymatic hydrolysate (Costantino Spa) 10, pH 6.8. In 300 mL flasks, medium was supplemented with 20 or 50 g/L glucose (G20-G50) or fructose (F20-F50), while trials performed with 1 L flasks employed glucose only (G20-G50). Carbon sources were sterilized separately from media (112 °C for 30 min) and added before inoculum.

All flasks were inoculated with a 10 % (v/v) amount of a 16-h-old culture grown in IC G20 medium for the biomass production trials with glucose and IC F20 for those with fructose. Cultures were incubated at 30 °C for 16 or 24 h under stationary conditions.

Zymomonas mobilis growth in fermenter

Trials were carried out in a 14-L fermenter (10 L working volume) (Omnitec, Sedriano, Italy) employing the IC G50 medium, sterilized directly in the fermenter; glucose was sterilized separately and added before the inoculum. Growth conditions were set as follows: temperature 30 °C, pH stat 5.5 with 1 M NH_4OH, 200 rpm, no aeration (nitrogen was bubbled for 10 min before inoculum), overpressure 0.15 bar, incubation time 9 or 16 h, inoculum 10 % (v/v) of a 16-h-old culture grown in IC G20 medium as reported previously.

Biomass growth determination

An aliquot (200 mL) of culture broth obtained at the end of incubation was centrifuged at 8,600 *g* for 20 min at 5 °C in a Beckman J2-21 centrifuge. The recovered cell pellet was washed with deionized water and centrifuged again under the same conditions. Dry cell weight (dcw) was determined after drying the cell pellet at 105 °C for 24 h.

Evaluation of leavening performance in a model dough

Gas production ability (mL/g dcw) was tested employing a procedure adapted from Burrows and Harrison (1959). Cell

sample (160 mg dcw) was suspended in 15 mL distilled water and then added to 20 g commercial Type 0 wheat flour (Manitoba), suitable for bread making. In the case of *Zymomonas*, glucose (2 g) was also added. The dough was made by mixing all ingredients (kneading done by hand with a spatula for 45 s) in a double chamber glass flask, connected to a water bath at 29±0.5 °C. The flask was connected with a graduated burette, filled with saturated NaCl solution (to minimize CO_2 dissolution), equilibrated with atmospheric pressure. The kinetics of gas production (mL) was followed by measuring the liquid level at appropriate intervals. Data were normalized per gram dcw.

In each trial, the time course of gas production was fitted by employing DMFit 3.0 software, to estimate gas production rate (mL/g dcw min), lag leavening phase duration (min) and total amount of gas evolved (mL/g dcw), according to Baranyi and Roberts (1994) (Eq. 1):

$$y(t) = y_0 + \mu_{\max} t + \frac{1}{\mu_{max}} \ln\left(e^{-vt} + e^{-h_0} - e^{-vt-h_0}\right) - \frac{1}{m} \ln\left(1 + \frac{e^{m\,\mu_{\max} t + {}^{1}\!/_{\mu_{max}} \ln\left(e^{-vt}+e^{-h_0}-e^{-vt-h_0}\right)} - 1}{e^{m(y_{max}-y_0)}}\right) \quad (1)$$

In the present paper, the equation was applied to model gas production, where $y(t)$=mL gas/g dcw; y_0= mL gas/g dcw present at t_0; $y_{\max}$= (mL gas/g dcw)$_{\max}$; $\mu_{\max}$ is the maximum gas production rate (mL gas/g dcw min); m is a curvature parameter to characterize the transition from the exponential phase; v is a curvature parameter to characterize the transition to exponential phase; h_0=v, as suggested by the authors when fitting situations other than bacterial viable counts.

Determination of gas composition

The volume of gas evolved was also monitored by using a manometric device (LEO 2, Keller, Winterthur, Switzerland). Gas composition was determined using a gas chromatograph (Master GC Analyzer DGA52, Dani, Cologno Monzese, Italy) equipped with two different capillary columns: (1) Hayesep Q column (3 m, 1 mm ID, 1/16″ OD, 80/100 mesh) that enables the separation of CO_2 from the other gases, which were separated with: (2) Molesieve 5 Å column (3 m, 1 mm ID, 1/16″ OD, 80/100 mesh) in H_2, O_2, N_2, CH_4. The carrier gas was helium. The temperatures were 40 °C for the oven, 250 °C for the injector and 200 °C for the detector. Detection was done using a thermal conductivity detector (TCD A). The volume of injected biogas was 250 μL. The calibration was performed with a standard gas composed of 24.79 % CO_2, 4.98 % H_2, 0.976 % O_2, 10 % N_2 and 59.254 % CH_4.

Analytical determinations

Sugar (maltose and glucose) consumption, as well as ethanol and other metabolites produced during *Z. mobilis* growth or leavening trials were determined through an HPLC system (L 7000, Merck Hitachi, Tokyo, Japan) equipped with RI and UV (210 nm) serially connected detectors, using a (300–7.8 mm) Polispher OA KC (Merck, Darmstadt, Germany) column, maintained at 30 °C and eluted with 5 mM H_2SO_4 at 0.4 mL/min. Culture broth (2 mL) or dough (1–2 g) samples, the last previously suspended in distilled water (about 10 mL), were centrifuged (Eppendorf 5804, 10,600 *g*, 10 min) and the supernatants obtained analyzed after appropriate dilution. Data related to molecules detected in the culture broth are expressed in terms of grams per litre (g/L), while those found in leavening trials are expressed as grams per 100 g flour.

Statistical analysis

Statistical analysis of the results was performed with SPSS software, version 21.0 (SPSS, Chicago, IL). *T*-test and one-way analysis of variance (ANOVA) were applied to the data. Differences between means were assessed on the basis of confidence intervals using the Tukey-b test at a level of significance of $P<0.05$. Data are represented as means±standard deviation.

Results

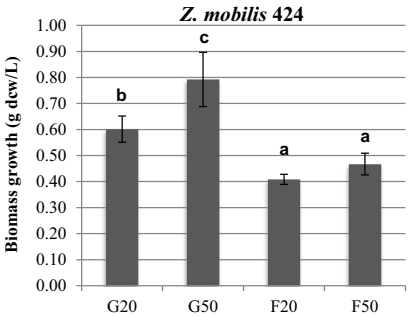
Biomass production in flasks

The two *Z. mobilis* strains were initially grown in 300 mL flasks, employing a culture medium (IC) formulation avoiding any yeast-derived compound, in order to preserve the yeast-free nature of the final biomass. Fructose or glucose were used at 20 or 50 g/L as carbon and energy source and cultures were incubated for 16 and 24 h, comparatively.

No significant differences ($P<0.05$) were found between biomass growth at 16 and 24 h (data not shown) in all the tested media and for both strains. The lowest biomass yields were obtained with fructose as energy source, in particular when set at 20 g/L (Fig. 1). Upon increasing fructose to 50 g/L the two strains performed differently: *Z. mobilis* 3580 increased biomass yield while *Z. mobilis* 424 did not.

In contrast, glucose gave the highest results in terms of biomass growth, and increasing glucose from 20 to 50 g/L resulted in a statistically significant growth increase (about 32 %) for both bacteria tested.

Trials performed in 1 L flasks (Fig. 2) with glucose (20 or 50 g/L) confirm the results obtained with 300 mL flasks: again, no difference in terms of biomass growth was observed for



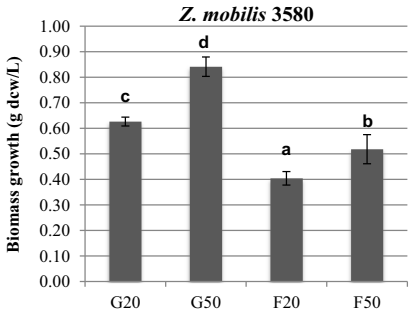


Fig. 1 Biomass growth (g dcw/L) of *Zymomonas mobilis* 424 (*left*) and 3580 (*right*) in IC medium with 20 or 50 g/L glucose (G20-G50) or fructose (F20-F50) in 300 mL flasks. Data were obtained by averaging *Z. mobilis* growth at 16 and 24 h incubation (means±standard deviation, $6<n<8$). Different *superscript letters* indicate significant differences (Tukey-b test, $P<0.05$)

cultivation times of 16 and 24 h (data not shown). *Z. mobilis* 3580 and 424 grew similarly at the two glucose concentrations tested, with higher yields (22 % and 28 %, respectively) when glucose was set at 50 g/L as compared to 20 g/L.

Comparing results obtained in 300 mL and 1 L flasks, we can in general observe a positive and significant (*t*-test, $P<0.05$) up-scaling effect, with a growth increase between 11 % (*Z. mobilis* 3580, 20 g/L glucose) and 16 % (*Z. mobilis* 424, 20 g/L glucose).

Zymomonas growth in fermenter

The next step comprised biomass cultivation of *Z. mobilis* (3580 and 424) in IC G50 medium in a 14-L fermenter. The incubation time was shortened, and culture samples analyzed at 9 and 16 h incubation. When downstream analysis was performed at 9 h, HPLC analysis evidenced for both strains a quantity of residual glucose (about 7 g/L) in the cultures, which disappeared at 16 h (Table 1). When prolonging the incubation time, biomass conversion yield ($Y_{X/S}$) decreased from about 3.5–4.0 % at 9 h to 3.0–3.1 %, while ethanol conversion yield ($Y_{P/S}$) increased from 42–46 % to nearly 50 %.

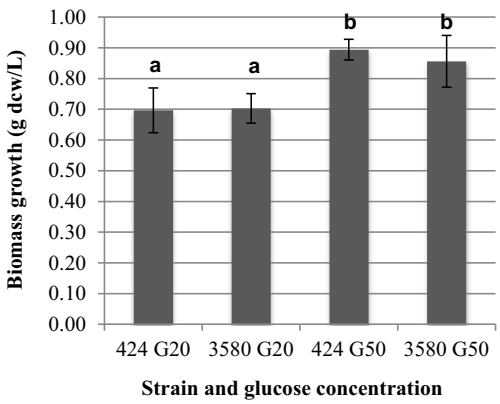


Fig. 2 Biomass growth (g dcw/L) of *Z. mobilis* 424 and 3580 in IC medium with 20 (G20) or 50 (G50) g/L glucose in 1 L flasks. Data were obtained by averaging *Z. mobilis* growth after 16 and 24 h incubation (means±standard deviation, $6<n<8$). Different *superscript letters* indicate significant differences (Tukey-b test, $P<0.05$)

The obtained data indicate a biomass production increase of about 60 % up-scaling the process from 1 L flask to fermenter.

Leavening performance in model system: *S. cerevisiae*

Dough leavening trials were first performed employing *S. cerevisiae* (control sample). Leavening ability was tested both in the absence of glucose (according to the procedure described by Burrows and Harrison 1959) and with glucose added in the dough, as performed for *Z. mobilis*. Results obtained employing the water displacement method were compared with those recorded with the manometric procedure coupled with gas analysis, to assess whether the gas production measured with the first method was equivalent to the CO_2 determined by the second procedure. The two methods provided similar results (Fig. 3) and, as expected, *S. cerevisiae* in presence of glucose produced a total amount of gas evolved higher than in its absence, about 1,000 vs 600 mL/g dcw in 180 min, respectively.

Time course of CO_2 production in each trial was fitted employing the DMFit 3.0 Excel add-in shareware package, useful for fitting sigmoid curves, to obtain CO_2 production rate (mL/g dcw min), lag leavening phase duration (min) and total amount of CO_2 evolved (mL/g dcw) as predicted by the Baranyi and Roberts model equation (1994). The results showed that glucose did not affect either the lag leavening

Table 1 Residual glucose (g/L) and biomass production of *Zymomonas mobilis* strains DSMZ 424 and 3580 (in terms of g dcw/L and Yx/s %) and ethanol production (g/L and Yp/s %) at 9 and 16 h of incubation. Trials were performed in 14-L fermenter. Data are reported as mean ($n=3$, CV in the range 5–8 %)

Strain		Glucose (g/L)	Biomass (g dcw/L)	Yx/s (%)	Ethanol (g/L)	Yp/s (%)
Z. mobilis 424	9 h	7.7	1.49	4.0	17.3	45.8
	16 h	0	1.42	3.1	22.6	49.7
Z. mobilis 3580	9 h	7.2	1.32	3.5	16.2	42.2
	16 h	0	1.36	3.0	20.1	44.2

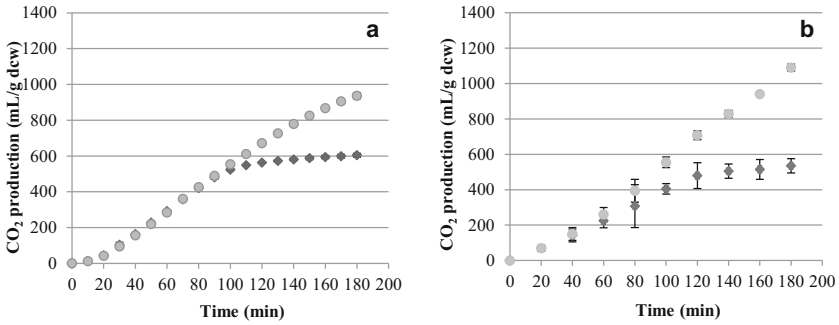


Fig. 3 Time course of CO_2 production (mL/g dcw) (means±standard deviation, n=3) in dough leavening trials performed with *Saccharomyces cerevisiae* with (*gray circles*) and without (*black diamonds*) glucose addition, evaluated through a water displacement method (**a**) and a manometric procedure coupled with gas analysis (**b**)

phase duration (7.8 min) or the gas production rate (6.4 mL/g dcw min), but only the final gas production levels (compared to line 1, Table 2). When employing glucose, the difference in CO_2 levels detected by the two procedures at longer leavening times (936 and 1,090 mL/g dcw) may be related to a compression effect created by the dough, which was not considered when applying the water displacement method. Due to its easier detection, we decided to use the volumetric procedure to monitor *Z. mobilis* leavening trials, aware of the possibility of underestimating gas production levels only at long incubation times.

HPLC analysis (Table 3) performed on dough samples collected at the beginning and at the end (180 min) of leavening trials evidenced that when glucose was added in the dough, *S. cerevisiae* used it up (almost 78 %), leaving maltose present in flour; instead, without glucose addition, *S. cerevisiae* used all maltose present into the flour. At 180 min, the dough samples prepared with and without glucose evidenced similar ethanol levels, at 1.2–1.3 g/100 g flour, respectively.

Leavening performance in model system: *Z. mobilis*

Figure 4 represents gas evolution during 180 min dough leavening trials with the *Z. mobilis* biomass samples produced in the fermenter (two strains, each of them collected after 9 or 16 h incubation time). Both strains evidenced better performance when biomass production step lasted for 9 instead of 16 h (Table 2), with a gas production rate statistically higher than *S. cerevisiae*. *Z. mobilis* cells grown for 16 h have a gas production rate not statistically different from those obtained by *S. cerevisiae*. Lag leavening phase was absent for *Z. mobilis* while present for *S. cerevisiae* (11 min).

HPLC analysis (Table 3) performed on dough samples collected at the end (180 min) of *Zymomonas* leavening trials evidenced that, as expected, maltose remained unconsumed. Moreover, biomass grown for 9 h was able to consume all glucose present in the dough, while dough leavened with 16 h-grown biomass evidenced a residual 2 % glucose. Also ethanol detected in dough leavened with 9 h-grown biomass was higher (4.3 and 1.5 g/100 g flour, respectively). HPLC analysis evidenced the presence of lactic and acetic acid at 180 min and, as for ethanol, in higher concentration in dough leavened by *Z. mobilis* grown for 9 h (0.26 and 0.06 g/100 g flour, respectively) than for 16 h (0.09 and 0.02 g/100 g flour respectively).

Table 2 CO_2 production rate (mL/g dcw min), lag leavening phase duration (min) and total amount of CO_2 evolved (mL/g dcw) (means± standard deviation, n=3), obtained fitting time courses of CO_2 production reported in Fig. 4 employing the DMFit 3.0 software (different superscript letters indicate significant differences, Tukey-b test, P<0.05)

Strain		CO_2 rate (mL/g dcw min)	Lag leavening (min)	CO_2 evolved (mL/g dcw)
Saccharomyces cerevisiae		6.4±0.3[a]	10.9±2.4	646±15[a]
Z. mobilis 424	9 h	8.9±0.6[b]	0	1,497±358[c]
	16 h	7.3±0.5[a]	0	964±212[b]
Z. mobilis 3580	9 h	10.9±1.1[c]	0	1,368±253[c]
	16 h	6.1±0.5[a]	0	831±91[b]

Discussion

The ability of *Zymomonas mobilis* to produce CO_2 has rarely been investigated, perhaps because this bacterium has a low molar growth yield that may increase biomass production costs compared with the more consolidated *S. cerevisiae*. Another technological limit of *Z. mobilis* vs *S. cerevisiae* is its ability to produce CO_2 only from glucose, fructose and sucrose; for this reason, a baked product leavened by *Z. mobilis* has to contain at least one of these sugars to allow the dough to rise significantly. These matters may have inhibited research into *Zymomonas* biomass application. To date, the only data reported in the literature regarding the use of *Z. mobilis* in bread making focused on the effects of NaCl and sucrose on the leavening ability of five *Z. mobilis* strains: the results showed that *Z. mobilis* strains are more sensitive

Table 3 Maltose, glucose, ethanol, lactic and acetic acids (g/100 g flour) present in dough samples at the beginning and at the end (0 and 180 min) of leavening trials performed with *S. cerevisiae* and *Z. mobilis* (means±standard deviation, n=3)

Strain	Time (min)	Maltose (g/100 g flour)	Glucose (g/100 g flour)	Ethanol (g/100 g flour)	Lactic acid (g/100 g flour)	Acetic acid (g/100 g flour)
S. cerevisiae −glucose	0	0.97±0.21	0.11±0.04	0	0	0
	180	0	0	1.18±0.12	0	0
S. cerevisiae +glucose	0	0.74±0.18	10.49±0.20	0	0	0
	180	0.70±0.05	2.35±0.15	1.32±0.20	0	0
Z. mobilis 9 h	0	0.81±0.12	10.29±0.21	0	0	0
	180	0.85±0.10	0	4.30±1.41	0.26±0.07	0.06±0.01
Z. mobilis 16 h	0	1.08±0.19	10.07±0.19	0	0	0
	180	0.82±0.13	0.2±0.05	1.52±0.50	0.09±0.06	0.02±0.01

than *S. cerevisiae* to the presence of NaCl in a dough (Oda and Tonomura 1994).

In the present study, we evaluated *Z. mobilis* growth performance in a medium formulated avoiding any yeast-derived compound, and adding glucose or fructose as energy source. The best condition was then up-scaled and the collected biomass was employed to test *Z. mobilis* leavening ability in a dough model system. We chose not to consider sucrose in the initial screening, in order to avoid levan formation, as its synthesis would have further reduced biomass yield. Levan is an exopolysaccharide synthetized by *Zymomonas* on sucrose-based substrates (Lee and Huang 2000), and has applications in the food industry (De Oliveira et al. 2007) and in baked goods (Arendt et al. 2007) where it contributes to softening and antistaling of wheat breads (Jacob et al. 2012). Of course this interesting aspect will be investigated in future trials.

Biomass yields obtained in our yeast-free medium containing 20 g/L glucose (about 0.70 g dcw/L for both strains) are similar to those reported by other authors: Oda and Tonomura (1994) obtained 0.59 g dcw/ L with *Z. mobilis* 3580 (Z6) and 0.50 g dcw/L with *Z. mobilis* 424 (Z8); Zikmanis et al. (1997) obtained about 8 g/mol with *Z. mobilis* 3580, which corresponds to about 0.88 g dcw/L. Nevertheless, all these reported formulations contain yeast extract.

Results obtained in flasks demonstrate that biomass yield is about 50 % higher with glucose than with fructose, and confirm data reported by Lee and Huang (2000) that the more glucose present, the higher the biomass yield. However, biomass conversion yield ($Y_{X/S}$)—which represents how efficiently *Z. mobilis* converts substrate into biomass—decreases. Another interesting result is the positive up-scaling effect on biomass production, from around 0.85 g dcw/L in flasks to 1.40 g dcw/L in a fermenter (for both *Z. mobilis* strains), where incubation time was also shortened to 9 h, with positive implications both for biomass yield ($Y_{X/S}$) and dough leavening performance.

Taking into account the low molar growth yield of *Z. mobilis*, future investigation will address increased biomass yield, perhaps applying a fed-batch fermentation process while maintaining dough leavening ability.

As regards leavening trials, our results demonstrate that *Z. mobilis*, when glucose is present, is able to rise a dough, with similar or higher gas production rates (mL/g dcw min) and total amount of gas evolved (mL/g dcw) to *S. cerevisiae*. Glucose addition in leavening trials with *S. cerevisiae* produced only higher amount of CO_2 evolved but did not affect either the gas production rate or the lag leavening phase duration. These data were similar to those reported by Oda and Tonomura (1994), even if the dough composition was different: the latter authors reported leavening ability in terms of milliliters gas developed in 2 h per 10 g flour on a dry cell weight basis, and stated that *Z. mobilis* performed about 50 % better than *S. cerevisiae*. We obtained the following ratios: 49 % for *Z. mobilis* 424 and 70 % for *Z. mobilis* 3580 (9 h-grown biomass, fermenter).

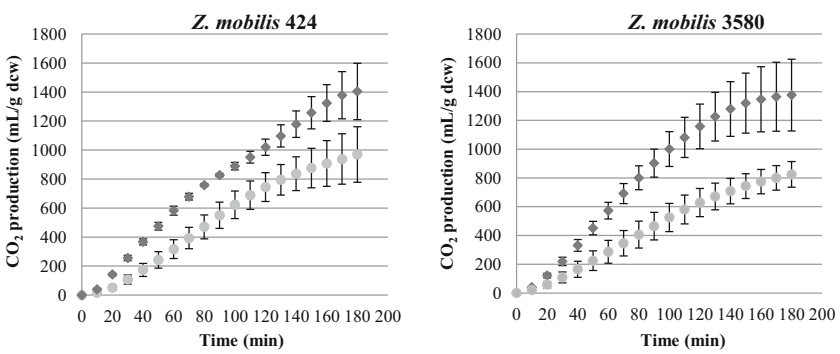


Fig. 4 Time course of CO_2 production (mL/g dcw) (means± standard deviation, n=3) in dough leavening trials performed with *Z. mobilis* DSMZ 424 (*left*) and 3580 (*right*). Biomass samples were collected after 9 h (*black diamonds*) or 16 h (*gray circles*) incubation in 14 L fermenter

Acetic and lactic acids found in *Z. mobilis* dough samples may give a slight acidic flavor to the final product as compared to a yeast-based dough (Oda and Tonomura 1994); nevertheless these compounds are also present in sourdough fermentations (Gobbetti et al. 2005). Of course, deeper studies have to be planned to investigate the sensorial and technological properties of doughs leavened by *Z. mobilis*, to assess the acceptability of the final product.

On the basis of these considerations, the study of new microbial populations in the area of leavened goods may be considered of actual relevance. *Z. mobilis* may provide a solution to the increasing incidence of food hypersensitivity where *S. cerevisiae* is involved. It should be noted that, in this research, yeast-derived compounds (i.e., yeast extract), commonly present in culture media, were avoided to preserve the yeast-free characteristic of the final leavened product. In summary, our results confirm the possibility of replacing *S. cerevisiae*, and the present research provides an innovative approach in the area of yeast-free leavened baked goods.

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