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Fermentative capacity of baker's yeast exposed to hyperbaric stress

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Abstract

Baker's yeast suspensions were incubated at different pressures (from 1 bar to 6 bar) and different gases [air, O_2 and a mixture of 8% (v/v) CO_2, 21% O_2 and N_2]. Raising the air pressure from 1 bar to 6 bar stimulated cell growth but had no effect on leavening ability or viability of the cells. A 50% reduction of the CO_2 produced in dough occurred with 6 bar O_2 which also stopped growth. The fermentative capacity of the cells was stimulated by the cells exposure to increased CO_2 partial pressure up to 0.48 bar.

Introduction

The main function of baker's yeast (*Saccharomyces cerevisiae*) in the bread dough is the production of CO_2 from sugars. Thus, the attainment of a high fermentative capacity is a crucial quality of the final baker's yeast. Highly aerobic culture conditions are used in the baker's yeast production to maximize the cell growth. However, traditional methods of providing high O_2 transfer rates, such as the increase of stirring and air flow rates, do not prevent O_2 limitation in high cell density cultures. Thus, the process of baker's yeast production usually incurs a decrease in the specific growth rate due to the limited O_2-transfer capacity of industrial bioreactors. These low-specific growth rates have a negative impact on the fermentative capacity of the yeasts (van Hoek *et al.* 2000). Previous work has shown that the utilization of an increased pressure of air or pure O_2 in the bioreactor is an effective way of improving the oxygenation of microbial cultures (Yang & Wang 1992) and can be applied to increase baker's yeast productivity (Belo *et al.* 2003). Nevertheless, no attempt has been made to analyse the effect of the hyperbaric stress on the leavening ability of the cells grown under increased pressure. Several environmental stress factors have been identified as determinant to the fermentative capacity of baker's yeast, such as osmotic stress (Hirasawa & Yokoigawa 2001, Trainotti & Stambuk 2001) and carbon and nitrogen starvation (Jorgensen *et al.* 2002, Thomsson *et al.* 2003).

In this work, the effect of the baker's yeast exposure to increased pressure of air, O_2 and CO_2 on the leavening ability of the cells was studied.

Materials and methods

Hyperbaric reactor

A stainless steel reactor Whithey 304-HDF4-300cc (Figure 1) was used to expose the yeast to hyperbaric environments and to measure the leavening ability of the cells.

Yeast, media and conditions

Commercial dried baker's yeast from DSM (Engedura) was used. Cell suspensions were prepared in the following culture medium: 5 g KH_2PO_4 l^{-1}, 2 g $(NH_4)_2SO_4$ l^{-1}, 0.4 g $MgSO_4 \cdot 7H_2O$ l^{-1}, 1 g yeast extract (Difco) l^{-1} and 5 g glucose l^{-1}. The medium was prepared in citrate buffer 50 mM, pH = 4. Cell suspensions of 10 g dry mass l^{-1} were incubated for 3 h in the hyperbaric bioreactor at 30 °C, 150 rpm at different values of pressure from 1 bar to 6 bar and gas

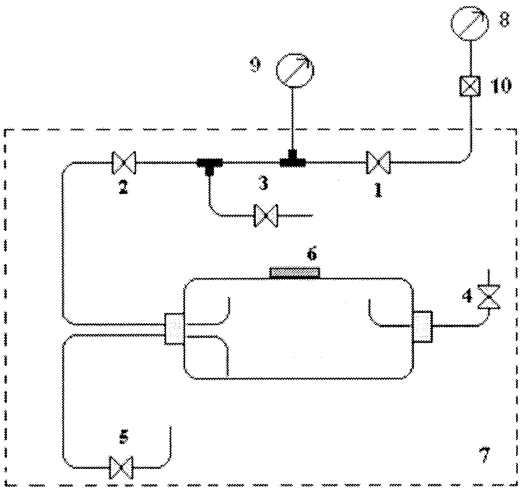

Fig. 1. Schematic representation of the hyperbaric reactor. The reactor is installed in a shaker thermostatic bath Neslab EX-600 (7) that enables agitation and temperature control. Each gas, from a gas container (8), is fed to the reactor through a gas filter of 0.45 μm porosity (10) and valves 1 and 2 (Whitey SS-ORS2). The pressure of the inlet gas sets operation pressure. The pressure transducer HD 9220 Delta OHM (9) measures the total pressure inside the reactor and overpressure is released by valves 3 or 4 (Whitey SS-ORS2). The reactor was charged with the cell suspensions or with the dough mixture through the hole with sealing cap on the top (6). Liquid samples were collected through valve 5 (Whitey SS-41S2).

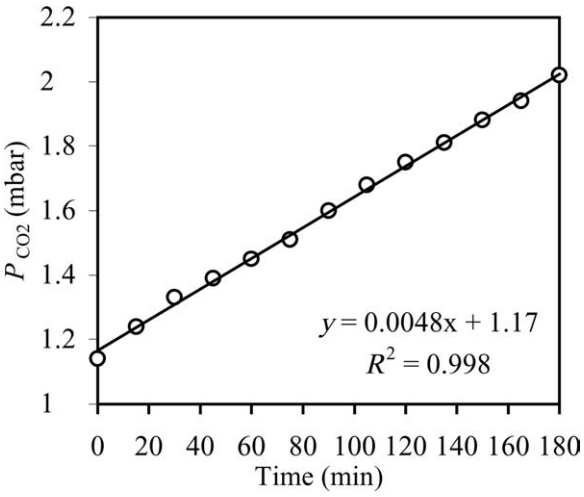

Fig. 2. Total pressure increase with time due to CO_2 evolved from dough of the control assay: cells not exposed.

composition [air, O_2 and a gas mixture of 8% (v/v) CO_2, 21% O_2 and 71% N_2].

Leavening ability determination

After incubation, yeast cells were harvested by centrifugation (5000 *g*, 10 min) washed with cold distilled water and recentrifuged. The yeast pellet was used to prepare the dough mixture. The ingredients of the dough were 100 g flour, 5 g glucose, 2 g yeast pellet and 62 ml distilled water (Hirasawa & Yokoigawa 2001). The ingredients were mixed for 15 min and placed into the cylindrical vessel, which was flushed with N_2. The leavening ability was measured by the rate of pressure rise inside the vessel (Figure 2). The increase of pressure is proportional to the CO_2 production. The pressure of carbon dioxide increased linearly with time through the analyzed period (2 h to 3 h).

Assays

Cell concentration of cell suspensions was determined turbidometrically at 620 nm and converted to dry cell mass per litre using a previously determined correlation factor. Cell viability was estimated by the Methylene Blue staining method (Jones 1987).

Results and discussion

Incubation of baker's yeast at increased air pressure stimulated cell growth (Figure 3a). The increase of O_2 availability on culture medium due to an O_2 partial pressure of 1.2 bar, using air at 6 bar or pure O_2 at 1.2 bar, increased the respiratory activity of the cells leading to faster growth than at low air pressure. These results are in accordance with the fed-batch growth of *S. cerevisiae* ATCC 32167 under hyperbaric air (Belo *et al.* 2003). The tolerance of O_2 toxicity up to 1.2 bar of baker's yeast was also shown by the insignificant difference (95% confidence level) in the viability of cells exposed to air at 1.2 bar and 6 bar, and to 1.2 bar of pure O_2 (Figure 3b).

According with the results in Figure 4, it is clear that is not the total pressure up to 6 bar, alone, that affects the leavening ability of baker's yeast. The CO_2 production rates in dough with baker's yeast exposed to air at 1.2 bar and 6 bar were quite close to the value obtained with cells not exposed (control). Thus, a 1.2 bar partial pressure of O_2 did not affect the leavening power of the cells. This was also confirmed by the experiment with 1.2 bar of pure O_2.

Also, the leavening ability of the cells was kept unchanged by the exposure to a hyperbaric environment of 6 bar of a mixture containing 8% (v/v) CO_2, 21% O_2 and N_2 compared to the control, despite reduction in cell growth and viability. Thus, the increase of CO_2 partial pressure up to 0.48 bar that can be attained

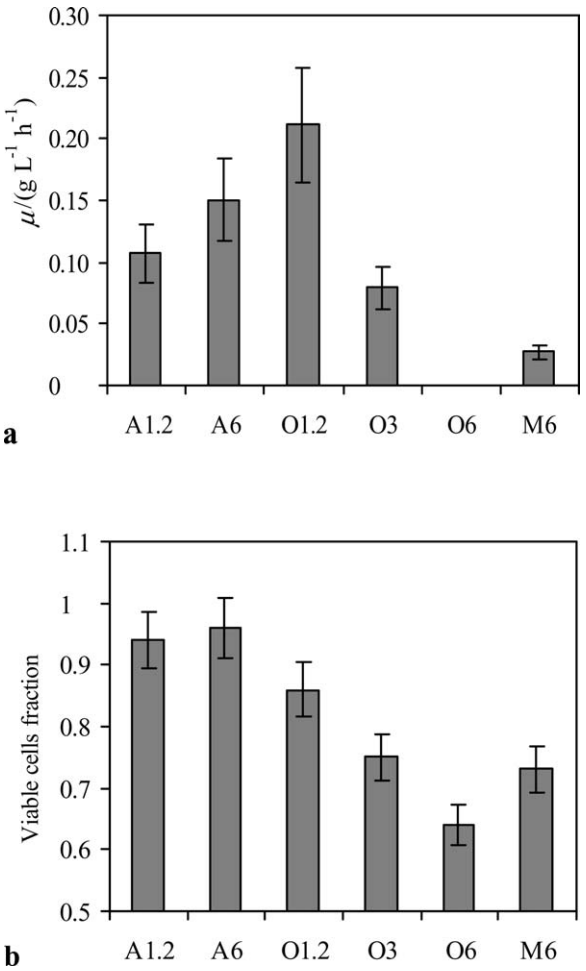

Fig. 3. Effect of gas pressure exposure for 3 h on the specific cell growth rate (a) and on the final fraction of viable cells (b) of baker's yeast. Condition labels: A – air, O – O_2 and M – gas mixture with 8% CO_2. Numbers in labels are pressure values (bar). Data are means ± 95% confidence interval.

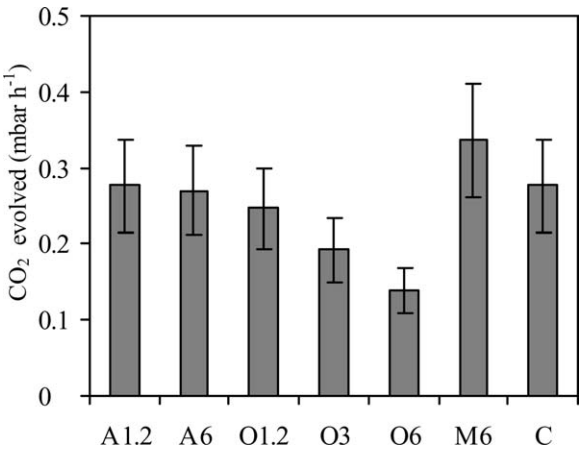

Fig. 4. Effect of gas pressure exposure of baker's yeast for 3 h on the rate of CO_2 evolution in bread dough in an anaerobic environment. Condition labels: A – air, O – O_2, M – gas mixture with 8% CO_2 and C refers to the control assay (cells not exposed). Numbers in labels are pressure values (bar). Data are means ± 95% confidence interval.

in yeast cultivation with hyperbaric air, will not have a significant impact on the fermentative capacity of baker's yeast.

When the hyperbaric incubation of cells was carried out with pure O_2 above 1.2 bar, growth, however, was drastically inhibited at 3 bar and completely stopped at 6 bar.

Previous work reported 8 bar of pure O_2 as a limit pressure for cellular growth of *S. cerevisiae* ATCC 32167 (Pinheiro *et al.* 1997). Here we found baker's yeast slightly sensitive to O_2 pressure.

Toxicity of O_2 affected the leavening ability of baker's yeast, since CO_2 production rate decreased by 36% and 54%, for O_2 pressure of 3 bar and 6 bar, respectively, compared to the experiment with 1.2 bar O_2 pressure.

Cell viability was reduced 1.34-fold by the increase in O_2 pressure from 1.2 bar to 6 bar, indicating that cell membrane is also affected by O_2 stress. This could have implications in sugars transport across the membrane, which has been reported has the major metabolic step affected by other stresses, such as NaCl stress (Trainottti & Stambuk, 2001, Rossel *et al.* 2002). On the other hand, O_2 effect on the leavening ability of yeasts could be an indirect effect of O_2 toxicity in cell growth, according with the reported influence of specific cell growth rate in the fermentative capacity of yeasts (van Hoek *et al.* 2000).

Further work is being carried on to identify the mechanisms of O_2 pressure effect on leavening ability of baker's yeast and to compare the baker's yeast response to other types of stress. Nevertheless, with the work here reported it is possible to conclude that hyperbaric air up to pressures of 6 bar, or pure O_2 up to 1.2 bar, can be applied for the aeration improvement of high cell density cultures of baker's yeast without affecting the fermentative capacity of the final product. The use of pO_2 above 1.2 bar is limited by the toxicity effects of O_2, which negatively affects the leavening power of baker's yeast.

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