



# AEROBIC AND ANAEROBIC CONDITIONS

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## ABSTRACT

*Saccharomyces cerevisiae* in complex medium were exposed to different values of total pressure (1 bar to 6 bar) at aerobic conditions, using air and pure oxygen, and at anaerobic conditions, using N<sub>2</sub> and CO<sub>2</sub>. The effects of pressure and gas composition on cell growth and ethanol production were evaluated. Also, cell morphologic changes were assessed by image analysis techniques.

## INTRODUCTION AND PURPOSE

Yeasts are widely used in industrial processes, such as the production of alcoholic beverages (beer and wine), biomass (baker's yeast and SCP), biomolecules (proteins, enzymes, recombinant bioproducts, etc) and several biotransformations.

Yeast cells have to face with different environmental conditions of gas composition and pressure depending on the process. Also, the gas phase in contact with the culture media changes through time due the metabolic activity of the cells, which, in turn, affects the behavior of the cell. Thus, gas composition and pressure are important factors for controlling processes where yeasts are used.

The purpose of this work is the study of the effects of total pressure raise on the behavior of yeast cells for different type of gas, in order to simulate aerobic and anaerobic conditions.

## METHODS

**Microorganism:** The *Saccharomyces cerevisiae* strain ATCC 32167 was used. **Culture medium:** 5 g/L KH<sub>2</sub>PO<sub>4</sub>, 2 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.4 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O, 1 g/L yeast extract and 5 g/L glucose. The medium was prepared in citrate buffer 50 mM, pH=4.0.

**Batch growth:** Yeast cells were cultivated during 7.5 h in a stainless steel bioreactor (PARR 4563) with 400 mL of working volume, at 30 °C, 400 rpm of stirring rate and 1 L/min (measured at standard conditions) of inlet gas flow rate.

**Image analysis:** Image acquisition was conducted in an optical microscope (Diaphot 300, Nikon Corp.) with 400x magnification coupled with a black and white camera (Sony CCD AVC D5CE) and linked to a microcomputer by a frame grabber (DT3155, Data Translaction, Inc.). Traditional tools generally used for image processing were employed. Fully automated image treatment was performed with Matlab v. 6.1 (The Mathworks Inc.) package has previously reported (Coelho *et al.*, 2002). Objects were labeled enabling to extract individual properties, such as area, major axis length, minor axis length and orientation, among others.

**Cell size distribution:** Projected area of the objects analyzed in the images of the samples was used for the estimation of cell size distribution. Mother and daughter cells were separated employing the watershed algorithm presented in the Image processing Toolbox (Matlab v. 6.1).

**Bud cells:** Bud cells were discriminated from non-bud cells through a parameter called "elongation" (major axis length/minor axis length). This parameter was computed according to Pons and Vivier (1998). For the yeast strain ATCC 32167 an average elongation factor of 1.5 was used for bud determination.

## RESULTS

The effect of pressure raise up to 6 bar on cell growth strongly depends on the nature of gas used for pressurization (Fig.1). According with previous work (Belo *et al.* 2003) air pressure up to 6 bar (1.2 bar oxygen partial pressure) improves cell growth. However, when pure oxygen is used cell growth inhibition is observed. This inhibition increases with the increase of oxygen pressure, which is a consequence of the oxidative stress caused to the cells by high levels of dissolved oxygen in the culture medium.

Under anaerobic conditions, no differences were found in cell growth at 1 bar and 6 bar of nitrogen. On the contrary, the increase of CO<sub>2</sub> pressure from 1 bar to 6 bar, significantly reduced cell growth.

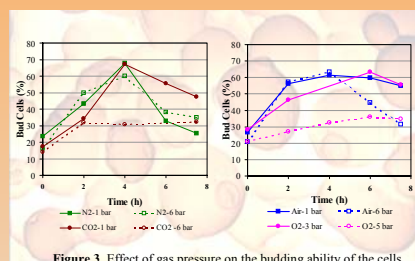


Figure 3. Effect of gas pressure on the budding ability of the cells

Cell inactivation by the increase of oxygen and carbon dioxide pressure was confirmed by the reduction of the percentage of bud cells through time (Fig. 3). The percentage of bud cells increases soon after inoculation, indicating growth processes and reaching its maximum value at the late exponential phase of growth. This is a common feature in all the experimental conditions tested with exception of 5 bar O<sub>2</sub> and 6 bar CO<sub>2</sub>.

## References:

- Pons M N And Vivier H (1998) Morphometry of yeast. In: Wilkinson M. H. F. Schut F. (Eds.) Digital Image Analysis of Microbes: Imaging, Morphometry, Fluorometry and Motility Techniques and Applications, John Wiley & Sons, England, pp 199-224.
- Coelho M A Z, Amaral A L, Belo I, Mota M, Coutinho A A P (2002) Classification of *Saccharomyces cerevisiae* morphology using image analysis. In: Proc. ESBES-4 Symposium, Life Science and Technology, Delft, The Netherlands, pp 96.
- Belo I, Pinheiro R, Mota M (2003) Fed-batch cultivation of *Saccharomyces cerevisiae* in a hyperbaric bioreactor. *Biotechnol. Prog.* 19, pp 665-671.

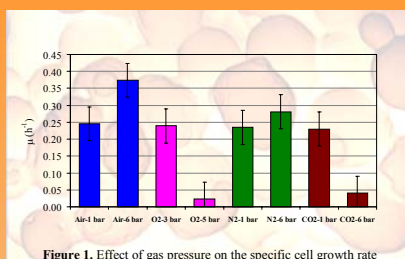


Figure 1. Effect of gas pressure on the specific cell growth rate

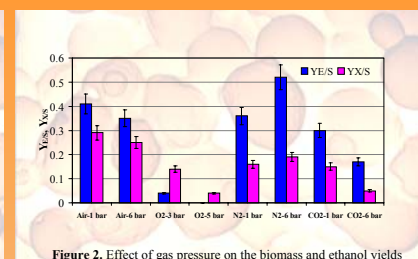
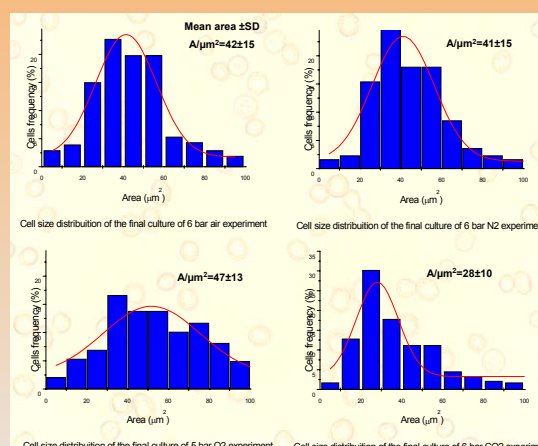


Figure 2. Effect of gas pressure on the biomass and ethanol yields

Ethanol production is strongly affected by oxygen and carbon dioxide pressure raise (Fig. 2). A higher level of inhibition of metabolic activity of the cells was found for oxygen exposure than for carbon dioxide. On the other hand, a 6 bar of CO<sub>2</sub> caused a 2.8-fold reduction of the ethanol yield compared with the value obtained for 6 bar of N<sub>2</sub>. Thus, the gas nature is also determinant for the effect of pressure on fermentation productivity.



The mean area of the initial cultures used in all the experiments was 33 ± 12 (μm<sup>2</sup>).

An increase in the cell size distribution was obtained for almost all of the gases and pressures tested, from the beginning to the end of the experiment (7.5 h).

This is in accordance with the cell growth retardation at the stationary growth phase.

The decrease of cell size found for the final cell culture exposed to 6 bar CO<sub>2</sub> could be explained by the cell growth inactivation in combination with cell membrane damage. CO<sub>2</sub> compression and decompression inside the cell may be responsible for the cell membrane leakage, which in turn, leads to the loss of intracellular biomolecules of low molecular weight with the consequent cell compression.