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Saccharomyces cerevisiae Morphology under Hyperbaric Gases

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1. Summary

The effects of hyperbaric stress on the morphology of *Saccharomyces cerevisiae* were studied in batch cultures under pressures between 0.1 MPa and 0.6 MPa and different gas compositions (air, O_2, N_2, or CO_2), covering aerobic and anaerobic conditions. A method using automatic image analysis for cell classification based on their morphology was applied to experimental data. Cell viability was assessed through the Methylene Blue staining method and the percentages of viable and non-viable cells were also estimated using digital image processing. The results show that the effect of pressure on cell activity strongly depends on the nature of the gas used for pressurization. While nitrogen and air to a maximum of 0.6 MPa of pressure were innocuous to yeast, oxygen and carbon dioxide pressure caused cell inactivation, which was confirmed by the reduction on the number of budding cells with time and also a decrease in the average cell size (0.6 MPa CO_2). A model taking into account cell viability reveals the opposing effects between oxygen availability and the baric and oxidative stresses present on the system. It is shown that cell viability in general is not constant during the experiments but strongly depends on the environment.

Keywords: *Saccharomyces cerevisiae*, pressure, viability, image processing analysis, hyperbaric stress

2. Extended Abstract

The yeast *Saccharomyces cerevisiae* is one of the most important microorganisms employed in industry. Growth rate, mutation, and environmental conditions affect yeast size and shape distributions but, in general, the influence of spatial variations in large-scale bioreactors is not considered. Many differences found between laboratory and industrial behavior can be partially explained by different environmental conditions, especially when gas solubility is an important parameter, since it is a function of the local position within the reactor. As a consequence, analysis of pressure effects in cell physiology and morphology must be considered. Both O_2 and CO_2 partial pressures are directly involved in yeast metabolism, the former being an essential nutrient for cell respiration whereas CO_2 is a product of cellular metabolic activity. Thus, batch studies with pure air, oxygen, nitrogen or carbon dioxide were performed, covering aerobic and anaerobic conditions often used in fermentation technology. Besides metabolic analysis of hyperbaric stress on yeast cells, a further insight was made into cell morphology and viability changes caused by different gases at moderate pressures.

Experimental conditions: *S. cerevisiae* ATCC 32167 was grown in a medium comprising 0.4 g l^{-1} $MgSO_4 \cdot 7H_2O$, 2.0 g l^{-1} $(NH_4)_2SO_4$, 5.0 g l^{-1} KH_2PO_4, 1.0 g l^{-1} yeast extract, and 5.0 g l^{-1}

glucose (pH 4.0). Hyperbaric experiments were carried out in a 600-ml stainless steel reactor (Parr 4563; Parr Instruments) at 30°C and 400 rpm. Compressed pure gas was continuously sparged into the culture medium at a flow-rate of 1 l min^{-1} (measured at standard conditions of pressure and temperature). The reactor was connected to different bottles containing the pure gases (air, oxygen, N_2 or CO_2). The operating pressure was set by manipulation of the pressure of the inlet gas and the regulatory valve position in the exit gas line. The reactor was equipped with a pressure transducer to monitor total internal pressure. The initial yeast concentration was 0.2 g l^{-1}. Cell concentration was estimated through optical density (620 nm), previously correlated to dry weight determination. Glucose was measured by the 3,5-dinitrosalicylic acid method. Ethanol was quantified by HPLC. Cell viability was determined through the Methylene Blue staining method and the amount of viable and non-viable cells were estimated using digital image processing. Further details about images acquisition and analysis procedure can be found in Coelho *et al.* (2004) and Coutinho *et al.* (2005).

Main Results and Discussions: The effect on cell growth of pressure up to 0.6 MPa strongly depends on the nature of the gas used for pressurization. An increase in air pressure to 0.6 MPa leads to a considerable increase (52%) in cell growth rate. On the other hand, the influence of an oxidative environment determined by a pure oxygen atmosphere of 0.3 MPa or above, drives the cell response in the opposite direction. Furthermore, an increase in oxygen pressure from 0.3 MPa to 0.5 MPa induced a drastic decrease (90%) in cell growth rate. Due to cell inactivation, no ethanol was produced, and glucose remained in the medium at a concentration near to the initial value. Under anaerobic conditions, different effects were also obtained for N_2 and CO_2 environments. An increase in N_2 pressure to 0.6 MPa did not affect cell growth, and slightly enhanced ethanol production. Thus, as far as fermentation process are concerned, the size of the reactor and local variations in pressure due to hydrostatic liquid pressure (to a maximum of 0.6 MPa), should not interfere with process performance if nitrogen is used as sparging gas. On the other hand, a CO_2 environment of 0.6 MPa inhibited the fermentation process.

The morphology of the cells seems to be intimately correlated with their physiological state. Since no significant increase in the percentage of bud cells over time was obtained for O_2 and CO_2 at high pressure, it can be stated that these conditions lead to inhibition of cell division. Moreover, experimental conditions with high substrate consumption rates led to a decrease in bud cell percentage. Under CO_2 and O_2 at high pressure, the bud cell percentage remained constant until the end of the process, indicating that cells were kept in the lag phase. In spite of the similarity between the effects of high-pressure O_2 and CO_2 on bud formation, a cell size decrease was found in the case of the final culture exposed to 0.6 MPa CO_2.

The cell viability analysis along batch cultivation reveals a complex pattern relating opposite influences of the increase in oxygen content and pressure. In fact, the pressure increase leads to a favorable effect in cell viability until pressures of about 1.0MPa that can be related to an increment in oxygen availability at high pressure. At pressures higher than 1.0 MPa, the advantages of the increment in oxygen content are offset by the oxidative stress. For anaerobic conditions an increase of N_2 pressure did not affect cell viability at pressures up to 0.6MPa. The modeling approach proposed describes the effects of barometric pressures of different gases (air, O_2 or N_2) on the loss of viability, showing that the common assumption that cell viability is constant during the fermentation may hold in some cases but, in general, is not correct.

References

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