Hyperbaric bioreactors use with *Yarrowia lipolytica* cultures: cellular adaptation to hyperbaric conditions

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INTRODUCTION

Y. lipolytica is a non-conventional yeast, non-toxic, that can grow to very high-cell-densities[1]. It is most used in studies of the biodegradative pathways for a variety of hydrophobic compounds including alkanes, oils, and fatty acids[2] and thus for its capacity to produce lipid-degrading enzymes, such as lipases.

The amount of oxygen available in culture media to *Y. lipolytica* is an important parameter since this organism is strictly aerobic. Previous work demonstrated that hyperbaric air could be successfully applied to yeast cultivation, as a way of improving the oxygen transfer rate to aerobic cultures[3-5].

Cells in industrial bioreactors are often exposed to O_2 partial pressures higher than 210 mbar (corresponding to air at 1 bar). In many cases, increased O_2 partial pressure can be toxic to aerobic cultures and inhibit microbial growth and product formation[4]. During the reduction of molecular oxygen to water through acceptance of four electrons, reactive oxygen species (ROS) are generated, which may give rise to damage of enzymes, nucleic acids, or lipids[6]. Antioxidant enzymes, such as catalase and superoxide dismutase (SOD), constitute the primary defences of the cells against ROS because they are responsible to transform these species into nonreactive ones[7].

The aim of this work is to investigate whether increasing air pressures may lead to increasing biomass yields of *Yarrowia lipolytica* W29, without giving rise to oxidative stress. Thus, the ability of the strain to induce antioxidant enzymes as a response to increased oxygen partial pressure was also assessed. Moreover, this work reports an investigation into the influence of a pre-adaptation phase of cells to hyperbaric conditions on the lipase production by *Y. lipolytica* cells.

STRAIN AND MEDIA

Yarrowia lipolytica W29 (ATCC 20460) was grown in YPD medium. The production medium was composed of 6.7 g·L^{-1} yeast nitrogen base w/o aminoacids, 7 g·L^{-1} olive oil, 5 g·L^{-1}arabic gum and Tris-HCL 400 mmol·L^{-1} buffer, pH 7.2.

BATCH CULTIVATIONS

Pressure: from 1 bar to 6 bar;

Agitation: 400 rpm;

Air flow rate: 1 vvm

Bioreactor: 600 ml stainless steel stirred tank (Parr 4563, Parr Instruments, USA)

AIR EFFECTS ON CELL GROWTH

The application of 6 bar stimulated cell growth compared to the atmospheric conditions. An increase of the cell dry weight at 6 bar of 3.5- and 5-fold was obtained compared with the experiments under atmospheric pressure in the control essay and in the bioreactor at 1 bar, respectively.

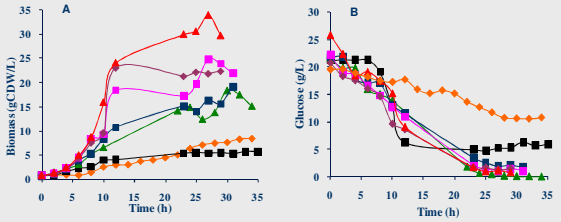

Fig. 1 Batch growth (A) and glucose consumption (B) of *Yarrowia lipolytica* at atmospheric pressure (♦) and in hyperbaric reactor under pressures of 1 bar (■), 2 bar (▲), 3 bar (■), 4 bar (■), 5 bar (♦) and 6 bar (▲).

In experiments under atmospheric pressure and 1 bar of air pressure, glucose was not totally consumed. On the other hand, the raise of air pressure up to 6 bar led to a completely consumption of glucose.

Table 1 Changes in biomass yield, specific growth rate and productivity with air pressure in batch experiments.

Air pressure (bar)	Control	1	2	3	4	5	6
$Y_{x/s}$ (gCDW /g) (%)	53.3	34.7	70.2	96.7	104.1	117.9	121.1
μ (h^{-1})	0.09	0.18	0.19	0.23	0.26	0.28	0.31
P (gCDW/L·h)	0.25	0.17	0.45	0.62	0.71	0.77	1.02

With 6 bar air pressure biomass yield by 56 % and 71 % compared with the experiments under atmospheric pressure (control) and 1 bar, respectively. Also, a 4.1-fold improvement in biomass productivity was obtained with the increase of air pressure up to 6 bar comparatively to the control.

An increase of 6-bar led to a 3.4- and 1.7-fold increase in specific growth rate under atmospheric pressure and 1 bar, respectively. Due to the high oxygen mass transfer rate, the cells had more oxygen availability in the medium, thus cells grew faster, and less time was necessary to obtain maximum cell concentration.

It is clear from these results that an increase of air pressure up to 6 bar might successfully be applied to the improvement of biomass production of *Y. lipolytica* W29.

AIR EFFECTS ON ANTIOXIDANT ENZYME ACTIVITIES

Superoxide dismutase specific activity was induced by air pressure increase to a maximum of 6 bar. An increase of the SOD specific activity at 6 bar (1.26 bar of oxygen partial pressure) of 53.4-fold was obtained compared with the experiments under 1 bar.

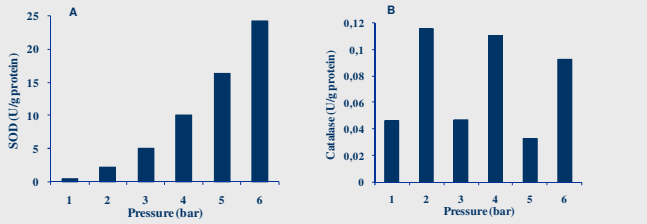

Fig. 2 Effect of air pressure on superoxide dismutase (A) and catalase (B) specific activities, in the final batch cell cultures (approximately 30 h of growth).

The SOD induction showed the cell sensitivity to high dissolved oxygen concentrations. However, as no cell growth inhibition was observed under pressurized conditions, it is quite safe to state that the cells of the strain used can cope with such high air pressure values up to 6 bar, that corresponds to a 6-fold increased in oxygen solubility in the medium.

The influence of total air and oxygen pressure increase on the catalase activity is not clear, thus it seems that this enzyme plays a minor role in the defensive mechanisms against the oxidative stress caused by oxygen partial pressure increase, for *Y. lipolytica* W29.

These results demonstrate that the raise of air pressure could be also applied to SOD production, once it is most induced.

AIR EFFECTS ON LIPASE PRODUCTION AND PRE-ADAPTATION

In order to investigate the influence of a pre-adaptation phase of cells to hyperbaric conditions on the lipase production by *Y. lipolytica* cells under increased pressure, assays were conducted in the pressurized bioreactor in which cells were pregrown on the bioreactor at normal and increased pressure following by a lipase production phase at normal and increased pressure.

An increase of the lipase activity and lipase productivity at 5 bar of 1.8-fold and 3.7-fold, respectively, was obtained compared with the experiments under 1 bar.

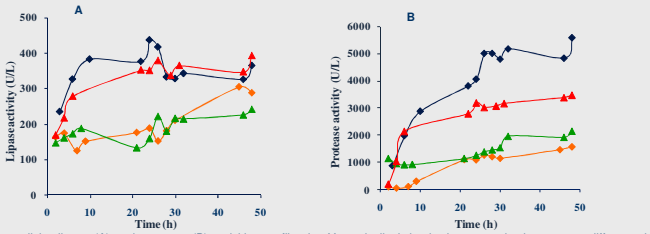

Fig. 3 Extracellular lipase (A) and protease (B) activities profiles by *Yarrowia lipolytica* in the pressurized reactor at different air pressures of growth and production, respectively: 1 bar and 1 bar (♦), 1 bar and 5 bar (♦), 5 bar and 1 bar (▲) and 5 bar and 5 bar (▲).

The lipase production at 5 bar with cells pregrown at the same air pressure was similar to the obtained with grown cells under 1 bar. This indicates that responses of the cells, in what lipase production is concerned, occur irrespective of the pre-culture pressure conditions. Therefore, it can be concluded that the *Y. lipolytica* cells can quickly respond and adapt to hyperbaric conditions and no need of long phases of hyperbaric stress adaptation is needed.

During the first hours of culture the protease activity was low, increasing gradually until the end of the cultivation time, suggesting that the decrease of the medium pH (data not shown) favours the production of an acid protease by yeast.

The highest value of protease production was reached at the same air pressure (5 bar) that the maximum lipase productivity was obtained but the pre-exposition of cells to increased air pressure seems to slight reduce protease activity.

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