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ENHANCED GROWTH OF *PICHIA PASTORIS* UNDER INCREASED AIR PRESSURE ON DIFFERENT CARBON SOURCES

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KEYWORDS

P. pastoris, increased air pressure, anti-oxidant enzymes.

ABSTRACT

Batch fermentations, in hyperbaric reactor, were performed to study the effect of increase total air pressure on the growth of *Pichia pastoris* CBS 2612 on different carbon sources and respective anti-oxidative cellular response.
Pichia pastoris strain was grown in glucose, glycerol and methanol media under total air pressure from 1 bar to 5 bar. In all the experiments, the cultures reached maximum cell density at 5 bar of total air pressure. A 3-fold increase on specific growth rate was obtained at 5 bar on glycerol compared to the value at 1 bar. Biomass yield was also enhanced by air pressure rise, for all carbon sources. With 5 bar air pressure biomass yield (g cells/g carbon) was 0.97, 1.53 and 1.86 whereas at 1 bar was 0.67, 1.72 and 0.77, respectively in methanol, glucose and glycerol media.
For all carbon sources tested, an increase of air pressure led to an enhance of malondialdehyde, a lipid peroxidation marker. It was also observed an induction of anti-oxidant enzymes, such as SOD, catalase and glutathione reductase, indicating that cells were able to respond to the oxidative stress caused by oxygen partial pressure increase.

INTRODUCTION

Pichia pastoris has many biotechnological applications. Two aspects of the species have contributed to its utility: (1) fermentation techniques were developed for maintaining extremely high cell densities in excess of 100 g/L dry weight, and (2) because *P. pastoris* assimilates methanol, the expression system is linked with alcohol oxidase, which is abundantly produced in the presence of methanol.
Glycerol is regularly used as the main initial carbon source in *P. pastoris* fermentations to increase the cell concentration. As the strongly repressing carbon source, theoretically, glucose has been considered impracticable for *Pichia* fermentation. Economically, glucose can be an ideal alternative growth substrate for pure glycerol (Mayer et al., 1999). However, it is possible to make use of crude glycerol, the main byproduct of biodiesel production, as the carbon source in bioprocesses with the methylotrophic *Pichia pastoris* (Çelik et al., 2008).
The high oxygen demand of methanol metabolism and cultivation at very high-cell-density makes oxygen supply a major parameter in *Pichia pastoris* cultivation (Cereghino and Cregg, 2000). Previous work demonstrated that hyperbaric air could be successfully applied to yeast cultivation, as a way of improving the oxygen transfer rate (OTR) to aerobic cultures (Lopes et al., 2009).
Methylotrophic yeasts possess a respiratory type of metabolism and during growth an accumulation of superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2) takes place (Santovito et al., 2002). Moreover, the raise of total air pressure led to an increase of oxygen partial pressure, which also generates reactive oxygen species (ROS). ROS are highly damaging to all biological molecules, including DNA, proteins and lipids. The catalase and superoxide dismutase enzymes play a key role in cellular defense against these reactive species (Moradas-Ferreira et al., 1996).
Although the host and vector system and the cultivation process have been developed, the use of hyperbaric air on *P. pastoris* fermentations as a way to improve the oxygen restriction is still limited. In the present work, we investigate whether increasing air pressures may lead to

increasing biomass yields of *P. pastoris*, growing with three carbon sources, without giving rise to unbalance oxidative stress. Thus, the ability of the yeast to induce antioxidant enzymes as a response to increased oxygen partial pressure was also assessed.

OPERATING CONDITIONS

P. pastoris CBS 2612 cells were pregrown in 250 mL Erlenmeyer flasks filled with 100 mL of YP with each one carbon source at 140 rpm, 30 ºC, and overnight. Batch cultivations were carried out using a 600 mL stainless steel stirred tank bioreactor (Parr 4563, Parr Instruments, USA), with 400 mL of each one carbon source medium, at 30 ºC, and 400 rpm. Compressed air was continuously sparged into the culture at an aeration rate of 1 vvm. The values of air pressure studied were 1 bar, 3 bar and 5 bar. An experiment in an Erlenmeyer flask (500 mL) with 200 mL of each medium, under atmospheric pressure and an agitation rate of 140 rpm was used as a control.

AIR PRESSURE EFFECT ON CELL GROWTH

According to the results, and regardless of carbon source, the rise of total air pressure leads to an increase in the final cell dry weight. In experiments with glucose, a 3.3- and 2- fold improvement in biomass production was obtained with the increase of air pressure up to 5 bar compared to the control and 1 bar, respectively. In essays with glycerol as a carbon source, an increase of the cell dry weight at 5 bar of 1.2-fold was achieved comparatively to the experiments under atmospheric pressure and in the bioreactor at 1 bar.

The oxygen availability increase imposed by pressure raise had a clear positive effect on methanol metabolism since the biomass production for an air pressure of 5 bar was enhanced 44.6 % and 29.2 % compared to Erlenmeyer flask and 1 bar, respectively.

The specific growth rate achieved in glucose medium was similar for all values of total air pressure tested, as well as in methanol medium. However, the value obtained with glucose as a carbon source was 71 % higher than in a methanol medium.

AIR PRESSURE EFFECT ON ANTIOXIDANT ENZYME ACTIVITY

Superoxide dismutase was detected in the presence of glucose, glycerol and methanol into the medium. However, the influence of increase of total air pressure was more significant in cells that were grown in glycerol as a carbon source. An increase of the catalase-specific activity at 5 bar (1.05 bar of oxygen partial pressure), in the glycerol medium, of 10.6-fold improvement was obtained compared with the experiments under 1 bar. The cells that were grown in methanol medium showed a 5.7-fold improvement in catalase activity with the rise of total air pressure from atmospheric pressure to 5 bar.

CONCLUSIONS

The methylotrophic yeast *P. pastoris* has proven to be able to grown either in glucose, glycerol and methanol under increased air pressure. However, the specific growth rate and yield biomass obtained in methanol media was much lower than those attained with two other carbon sources. For all carbon sources tested, an increase of air pressure led to an induction of anti-oxidant enzyme catalase.

REFERENCES

Çelik, E.; N. Ozbay; N. Oktar; and P. Çalik. 2008. "Use of biodiesel byproduct crude glycerol as the carbon source for fermentation processes by recombinant *Pichia pastoris*". Ind Eng Chem Res 47, 2985 – 2990.

Cereghino, J.L. and J.M. Cregg. 2000. "Heterologous protein expression in the methylotrophic yeast *Pichia pastoris*". FEMS Microbiol Rev 24, 45 – 66.

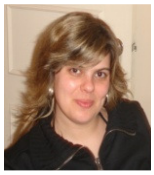
Lopes, M.; N. Gomes; M. Mota; and I. Belo. 2009. "*Yarrowia lipolytica* growth under increased air pressure: influence on enzymes production". Appl Biochem Biotechnol 159(1), 46 – 53.

Mayer, A.F.; K. Hellmuth; H. Schlieker; R. Lopez-Ulibarri; S. Oertel; U. Dahlems; A.W. Strasser; and A.P. van Loon. 1999. "An expression system matures: a highly efficient and cost-effective process for phytase production by recombinant strains of *Hansenula polymorpha*". Biotechnol Bioeng 63, 373 – 81.

Moradas-Ferreira, P., V. Costa; P. Piper; and w. Mager. 1996. "The molecular defences against reactive oxygen species in yeast". Mol Microbiol 19, 651–658.

Santovito, G.; B. Salvato; M. Manzano; and M. Beltramini. 2002. "Copper adaptation and methylotrophic metabolism in *Candida boidinii*", Yeast 19, 631 – 640.

AUTHOR BIOGRAPHIES



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