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

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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.

The high oxygen demand of methanol metabolism and cultivation at very high-cell-density makes oxygen supply a major parameter in *Pichia pastoris* cultivation. 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. Moreover, the energy and cost efficiencies of high-pressure fermentation for industrial application have already been demonstrated.

AIM

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.

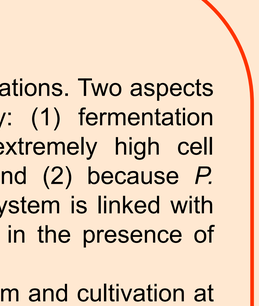
MATERIALS AND METHODS

➢ Strain: *Pichia pastoris* CBS 2612.

➢ Carbon sources: glucose (20 g/L), glycerol (10 g/L) or methanol (10 g/L).

➢ Aeration rate: 1 vvm.

➢ Air pressure: atmospheric (control trial) and 1, 3 and 5 bar (stainless steel stirred tank bioreactor, Parr 4563, Parr Instruments, USA).



RESULTS

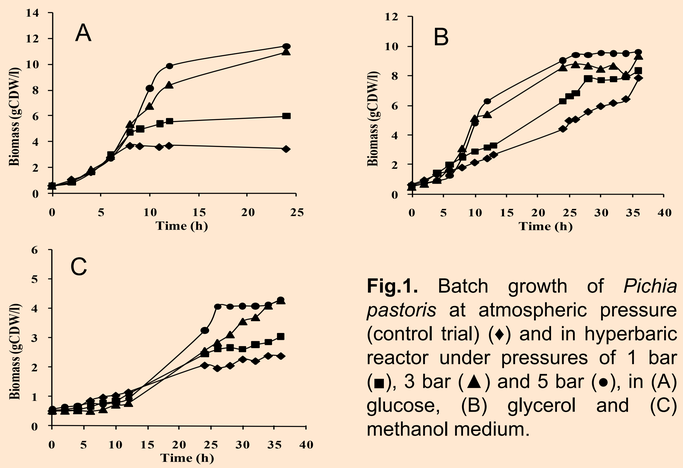


Fig.1. Batch growth of *Pichia pastoris* at atmospheric pressure (control trial) (♦) and in hyperbaric reactor under pressures of 1 bar (■), 3 bar (▲) and 5 bar (●), in (A) glucose, (B) glycerol and (C) methanol medium.

❖ With glucose, a 3.3-fold improvement in biomass production was obtained with the increase of air pressure up to 5 bar compared to the control.

❖ With glycerol, an increase of 1.2-fold in the cell dry weight at 5 bar was achieved comparatively to the experiments in the bioreactor at 1 bar.

❖ With methanol, the biomass production at 5 bar was enhanced 44.6 % and 29.2 % compared to Erlenmeyer flask and 1 bar, respectively.

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

		Flask	1 bar	3 bar	5 bar
μ (h^{-1})	Glucose	0.21	0.28	0.29	0.27
	Glycerol	0.06	0.07	0.22	0.23
	Methanol	0.06	0.07	0.08	0.08
$Y_{x/s}$ (g cells/ g carbon)	Glucose	0.40	0.77	1.48	1.53
	Glycerol	1.57	1.72	1.82	1.86
	Methanol	1.20	0.67	0.97	0.97

❖ The specific growth rate is maximum when cells are grown on glucose, followed by glycerol and the lowest value of this parameter was with methanol.

❖ The specific growth rate achieved in glucose medium was similar for all values of total air pressure tested, as well as in methanol medium. Probably, the highest value of OTR at 5 bar exceeded the demand for the cellular density present in the culture.

❖ With glycerol as carbon source, an increase to 5 bar led to a 3.8- and 3.3-fold improvement in specific growth rate under atmospheric pressure and 1 bar, respectively.

❖ In the glycerol medium the increase of total air pressure up to 5 bar resulted in an enhancement of 15 % and 8 % for the control and experiment under 1 bar, respectively.

❖ The reduced biomass yield per glucose in Erlenmeyer flask may reflect an altered metabolism, e.g., formation of by-products from the carbon source.

Table 2. Ethanol concentration in supernatant broth at 12 h of cultivation.

Carbon source	Flask	1 bar	3 bar	5 bar
Glucose	4.75	3.63	2.17	1.56
Glycerol	0.16	0.05	0.02	0

❖ At atmospheric pressure and 1 bar of total air pressure, glucose undertook fermentative metabolism; but pressure increase, reduces the ethanol formation.

❖ Ethanol was not detected in the broth supernatant of methanol medium, since P. pastoris is an obligate aerobic organism when grown on methanol and it cannot employ alternative metabolic reactions for methanol under oxygen limitation.

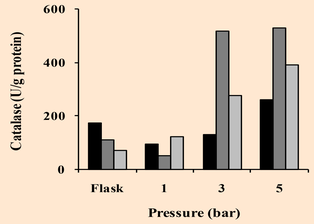


Fig. 2. Effect of air pressure on Catalase specific activity, in glucose (black bars), glycerol (grey bars) and methanol (white bars) medium.

❖ Catalase-specific activity was induced by air pressure.

❖ The catalase activity in the presence of glucose was lower than in the other carbon sources, due to glucose repression in methylotrophic yeasts of alcohol oxidase and catalase synthesis.