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EXPRESSION OF HETEROLOGOUS PROTEINS BY *PICHA PASTORIS* UNDER INCREASED AIR PRESSURE

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The *P. pastoris* expression system has gained acceptance as an important host organism for the production of heterologous proteins. Its expression system presents the advantage of the organism can grow to the desired cell density on glycerol as the carbon source and then on methanol for high level heterologous production. However, the oxygen limitation generally has a detrimental effect on the expression of foreign genes. The increased air pressure could be used to improve the oxygen solubility in the medium and to reach the high oxygen demand of methanol metabolism.

For the first time, two recombinant *P. pastoris* strains (KM71H/ pPICZ α A/frutalin and GS115/pPICZ/lacZ) producing frutalin and β -galactosidase, respectively, were used to investigate, the effect of increased air pressure on yeast growth and heterologous protein expression. Several trials were carried out in a stainless steel bioreactor (Parr 4563, Parr Instruments, USA) under total air pressure of 1 bar and 5 bar. The increase of air pressure up to 5 bar had a small effect on biomass production, but led to a 9-fold improvement in β -galactosidase specific activity compared to 1 bar. Also, the recombinant frutalin secretion was enhanced by the increased air pressure up to 5 bar.

The protease specific activity reached at 5 bar was 1.5 times lower than that obtained at 1 bar. This result revealed that the use of increased air pressure up to 5 bar provided optimal conditions for reduction of the proteolysis that occurred on frutalin secretion at atmospheric pressure.