

## Valorisation of glycerol by *Pichia pastoris* under increased air pressure

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*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 cell concentration. The recent abundance of crude glycerol, coming out as the main byproduct of biodiesel production, made possible the use of crude glycerol as the carbon source for bioprocesses with the methylotrophic *Pichia pastoris*. In 2009, 276 biodiesel plants were operating in Europe. The rapidly expanding market for biodiesel is decreasing the cost and increasing the glycerol availability, as typical biodiesel production processes generate around 10 % (wt) glycerol of the total amount of biodiesel produced.

Batch fermentations were performed in a hyperbaric reactor to study the effect of increasing total air pressure on *Pichia pastoris* CBS 2612 growing on pure glycerol, crude glycerol and methanol.

In the experiments with pure glycerol, a total pressure of 5 bar led to a specific growth rate 3-fold higher than the obtained under 1 bar. Also with crude glycerol, a 4-fold improvement in specific growth rate was obtained in the experiment at 5 bar compared to the control trial. Biomass yield was also enhanced by air pressure rise, for all carbon sources. Under 5 bar biomass yield (mass of cells per mass of carbon) was 0.97, 1.86 and 3.65 whereas at 1 bar was 0.67, 1.72 and 0.9 respectively in methanol, pure glycerol and crude glycerol media.

The current low cost of crude glycerol from the biodiesel production together with the present results shows the possibility of producing heterologous proteins using hyperbaric air with high productivity and at reduced costs.