Air pressure effect on growth behaviour of two different *Kluyveromyces* strains

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Keywords: Pressure, *Kluyveromyces*, yield, growth

ABSTRACT

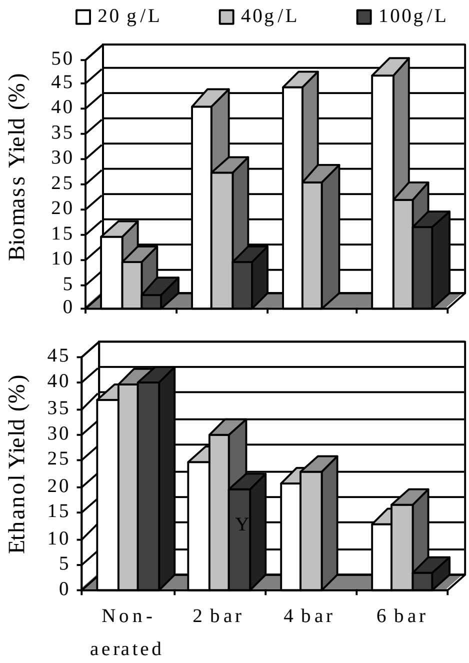
In the past decade, yeasts other than *Saccharomyces cerevisiae* have gained industrial interest. Examples are the lactose-utilising species *Kluyveromyces lactis* and *K. marxianus*. This type of yeasts are often proposed for reducing the BOD of cheese whey and other dairy plant wastes, for the production of flavours, enzymes and antibodies as well as for the expression of proteins. Their application in large-scale fermentations may be common industrial practice before the end of this century [1]. The use of pressure as a way of enhancing oxygen transfer rate to bioreactors can be investigated because oxygen is a major growth limiting factor in high density aerobic cultures. In biological processes the traditional way of improving oxygen transfer rate, by increasing stirring rate, has several limitations, like power consumption and cell damage, due to mechanical effects. The attempt to improve bioreactor performance has therefore been directed toward a reduction of the mechanical stress responsible for the cell inactivation [2]. The effect of increased air pressure on the biomass yield and ethanol yield of two *Kluyveromyces* strains was investigated. *K. marxianus* ATCC10022 is a lactose-fermenting strain whereas *K. marxianus* CBS 7894 has a Kluyver-effect for lactose. For *K. marxianus* ATCC10022 the air pressure increase of 2 bar, led to a 3 fold increase in biomass yield. It was also possible to enhance ethanol oxidation of cell yeasts by increasing air pressure. Batch cultures of *K. marxianus* CBS 7894 exhibited a different growth behaviour as *K. marxianus* ATCC10022: its metabolism was always oxidative and ethanol was never produced. With the increase of air pressure it was possible to increase biomass yield as well as the specific growth rate. On the other hand, as far as oxidative stress is concerned, antioxidant enzymes, such as superoxide dismutase, catalase and glutathione reductase, were not at high activities levels suggesting that there were no toxic effects on yeast cells for the studied pressures.

MATERIALS AND METHODS

Batch cultivations of *Kluyveromyces marxianus* ATCC10022 (“Kluyver-negative”) and CBS 7894 (“Kluyver-positive”) were carried out using a cylindrical pressure reactor with a total volume of 300 ml. The working volume was of 150 ml. The mineral medium consisted of: 5 g KH_2PO_4/L, 1.2 g $(NH_4)SO_4$/L, 0.4 g $MgSO_4.7H_2O$/L and 1 g yeast extract/L. The temperature was controlled at 30 ºC and agitation at 150 spm. The air feeding rate was of 150 ml/min (1 vvm). Three different air pressures: 2, 4 and 6 bar and three different lactose concentrations: 20, 40 and 100 g/L were studied. Non-aerated experiments were made in an Erlenmeyer flask under atmospheric pressure (1 bar), without air introduction. Growth was measured by optical density at 620 nm and converted to cell dry weight/L. Lactose was measured by determination of reduction sugars using the 3,5-dinitrosalisilic acid (DNS) method. Ethanol production was determined by HPLC.

RESULTS AND DISCUSSION

1. “Kluyver-negative” yeast: The use of increased air pressure to improve oxygen transfer rate in the culture was investigated with different lactose concentrations. In Figure 1 it is possible to observe that a small increase in absolute air pressure, to 2 bar, led to a 3 fold increase in biomass yield, for all lactose concentrations. Further increases in air pressure did not give more significant increases in biomass yield.



This *Kluyveromyces* strain seems to have an inhibitory effect for high lactose concentrations. With 100 g/L, cells consumed only 40% of it’s initial lactose concentration. However, with low lactose concentrations (20 and 40 g/L), this strain metabolised the substrate till its exhaustion producing biomass and ethanol. In all the experiments oxidoredutive metabolism was observed but with the increase in the partial oxygen pressure in the culture it was possible to decrease ethanol yield. Specific growth rate had a little decrease with pressure. Similar results for *Pseudomonas fluorescens* till a total pressure of 5 bar, were obtained by Onken [3]. From these results it seems clear that the increase in air pressure did not cause an inhibitory effect on yeast growth.

Fig. 1. Effect of air pressure on biomass and ethanol yield for different lactose concentrations.

Typical batch growth curves for lactose concentrations of 20 g/L and 40 g/L were found for this yeast. During a first phase cells consumed lactose, which was metabolised oxidoreductively, in a second phase ethanol was metabolised oxidatively. On the contrary, in experiments without pressure the ethanol produced was not oxidised by yeast cells. Figure 2 shows ethanol behaviour with the pressure: as pressure increased, ethanol production decreased. Pressure effects on ethanol production were more significant with an initial lactose concentration of 100 g/L. For 6 bar, practically no ethanol was produced, whereas with, 2 bar a high ethanol concentration was obtained and less biomass produced.

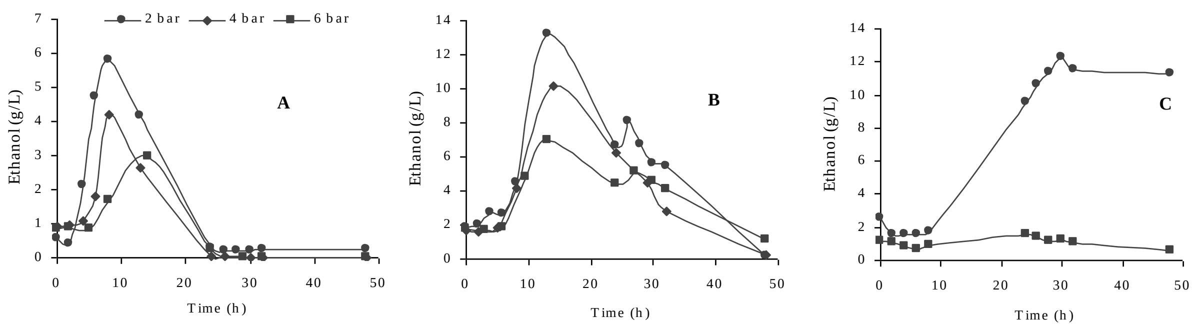
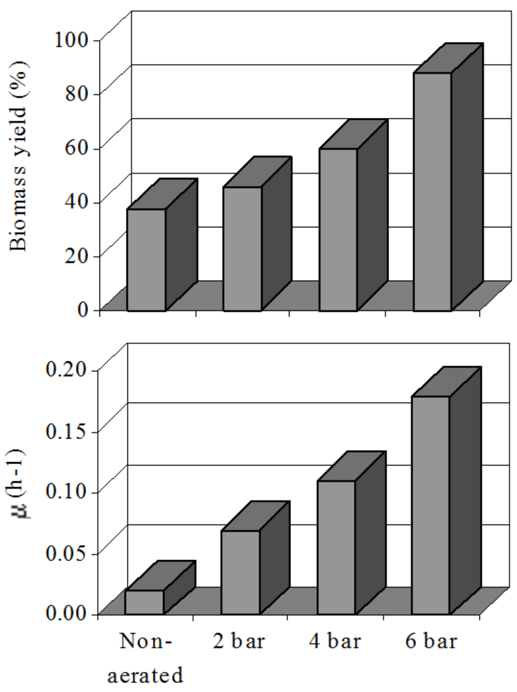


Fig. 2. Effects of air pressure on ethanol concentration for different lactose concentrations (A) 20 g/L, (B) 40 g/L and (C) 100g/L.

2. "Kluyver-positive" yeast: This yeast, *Kluveromyces marxianus* CBS 7894, exhibits a Kluyver effect for lactose and it was chosen as a model organism to investigate the application of pressure as a way of enhancing the oxygen transfer rate in the culture, without the production of ethanol.



In all experiments, including those without air introduction, oxidative metabolism occurred, ethanol was never produced, confirming its Kluyver-effect for lactose. Figure 3 shows the air pressure effects on biomass yield. Unlike the previous *Kluyveromyces* yeast, a small increase in air pressure, 2 bar, did not have a significative increase on biomass yield. An increase of 6 bar led to a 2 fold increase on biomass yield. The influence of air pressure on cell growth is shown in Figure 3, where the increase in specific growth rate with air pressure is evident. With pressurisation, maximum biomass concentration increased from 4 to 16 g dry cell/L. From these results it is clear that pressure had no inhibitory effects on the growth of this

Fig. 3. Effect of air pressure on biomass yield and specific growth rate for an initial lactose concentration of 40 g/L.

yeast and may be a way of eliminating oxygen limitation, leading to high biomass productivities.

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