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The Effect of Magnesium Ions during Beer Fermentation

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Abstract

When cells of Saccharomyces cerevisiae *ale strain C1028 were grown aerobically, ethanol production displayed a hyperbolic increase over a limited range of magnesium concentrations up to 700 μM. Entry of cells into the stationary growth phase and the time of maximum ethanol and minimum sugar concentrations correlated with a period of maximum Mg^{2+} concentration in the growing media. It is suggested that magnesium accumulation by yeast cells may be usefully exploited in biotechnology concerned with the production of beer.*

Keywords: *Yeast cells, magnesium ions, beer fermentation, ethanol production, hyperbolic increase, stationary growth phase, glucose consumption.*

Introduction

Magnesium ions play essential roles in the growth and metabolism of yeast cells. During beer fermentation Mg^{2+} ions are required as cofactors of the activity of key glycolytic and alcohologenic enzymes. Magnesium also plays a role in protecting yeast cells against environmental stresses during fermentation, such as those caused by ethanol (Dasary *et al.* 1990; Dombek and Ingram 1996), high temperature or high osmotic pressure (D'Amore *et al.* 1988). Indeed, Mg^{2+} deficiencies in the yeast fermentation broth are primarily responsible for the decline in yeast fermentation activity (Dombek and Ingram 1996).

A general stimulation of ethanol production by yeast can be seen when complex organic feed-stocks like molasses, wine must, or malt wort are supplemented with Mg^{2+}, indicating that such media may be deficient in available Mg^{2+} for optimal fermentation (Walker *et al.* 1996). Therefore, metabolic utilization of Mg^{2+} ions by yeast cells appears to be a prerequisite for the achievement of maximum fermentative activity.

The metabolic demands by yeast for Mg^{2+} during fermentation were investigated and are discussed in this paper.

Materials and Methods

Organisms, Media and Culture Conditions

The yeast employed in this study was industrial ale strain (C1028, UK). Active colonies of the yeast were grown on yeast peptone dextrose (YPD) agar slopes at 30°C for 24 hrs and then maintained at 6°C. Experimental fermentation was carried out by inoculating yeast cells in YPD media in which agar was omitted and the level of glucose was increased to 70 g/L. The calcium concentration was constant at 0.1 μM, but the magnesium concentration varied from zero up to 700 μM, as follows:

24 hr, 100 μM	120 hr, 500 μM
48 hr, 200 μM	144 hr, 600 μM
72 hr, 300 μM	168 hr, 700 μM.
96 hr, 400 μM	

In order to generate suitable inocula for experimental cultures 10 mL sterile water was added to YPD medium in an Erlenmeyer flask. This primary inoculum was incubated overnight at 30°C with shaking at 180 rpm prior to transfer to 400 mL fresh YPD medium. Fermentation was carried out at 6°C with a working volume of 400 mL.

Cell Numbers

Yeast cell colonies were determined using a counter (Stuart Scientific, UK). Cell density was measured using an absorption spectrophotometer (Spectronic, Genesys 5) at 600 nm. Ethanol was measured using an ebulliometer (Salleron Dujhardin, France). The residual glucose concentration in the yeast culture supernatants was measured by a refractometer (TAMCO, Japan).

Results and Discussion

The influence of magnesium on the fermentation metabolism of *Saccharomyces cerevisiae* ale strain C1028 was measured at the level of ethanol production. Both ethanol production and glucose consumption were dependent on the availability of Mg^{2+} ions in the yeast growth medium (Figs. 1 and 2). The concentration of magnesium affected fermentation by an increase in the number of yeast cells (Fig. 3). Concentration of >700 μM Mg^{2+} did not affect fermentation. Over the limited range of Mg^{2+} studied the fermentation increased due to the capacity of individual cells to augment ethanol production and produce an increase in cell numbers.

The fact that yeast cells accelerate fermentation as more Mg^{2+} are available raises the problem of cell uptake of magnesium and its intracellular concentrations. As seen from Fig. 1, concentrations of 600 μM and 700 μM magnesium result in ethanol production which is twice as high compared with control values. Glucose consumption is about 1.5 times more intensive in the presence of increasing amounts of magnesium in the range from 300 to 700 μM magnesium (Fig. 2). Yeast cell growth is also affected by high magnesium concentrations (600 and 700 μM). The results of the present work show that at these concentrations cell density is three times higher compared with the controls (Fig. 3).

Saltukoglu and Slaughter (1983) stated that yeast cells absorbed a constant amount of Mg^{2+} per cell as long as the medium contained this ion. However, under limited Mg^{2+} conditions the yeast cells take up magnesium in proportion to its availability. Recently, Walker and Maynard (1997) indicated that Mg^{2+} utilization correlated with periods of maximal ethanol accumulation. They showed that Mg^{2+} transport in industrial yeast strains was potentially useful as a means of regulating fermentative activity by physiological adaptation through Mg^{2+} conditioning of seed inocula prior to alcoholic fermentation.

The mechanism of active Mg^{2+} transport through the yeast plasma membrane remains to be elucidated. Nevertheless, the current study serves to emphasize the close relationship between fermentation and Mg^{2+} demand in *S. cerevisiae.*

The results of the present investigation suggests that Mg^{2+} accumulation by yeast cells may be used in biotechnology concerned with production of ethanol, particularly in beer production.

References

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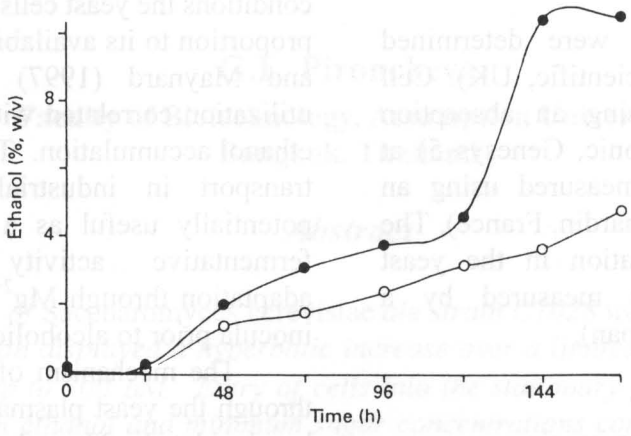

Fig. 1. The influence of Mg^{2+} concentrations from 100 to 700 μM on ethanol accumulation.
Legend: o = control fermentation without added Mg^{2+}
● = fermentation in the presence of increasing amounts of Mg^{2+} from 100 to 700 μM

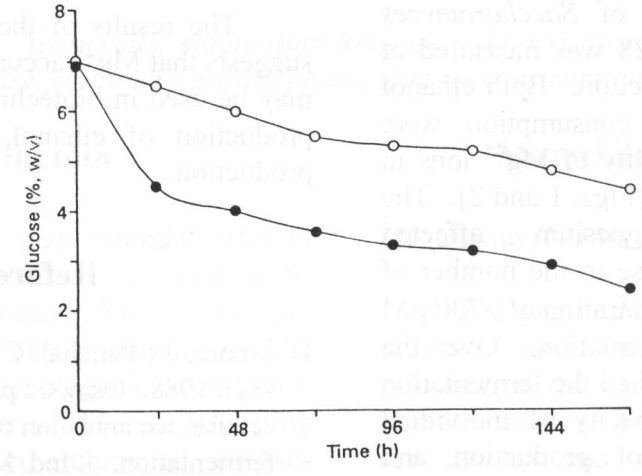

Fig. 2. The influence of Mg^{2+} concentrations from 100 to 700 μM on glucose consumption.
Legend: o = control fermentation without added Mg^{2+}
● = fermentation in the presence of increasing amounts of Mg^{2+} from 100 to 700 μM.

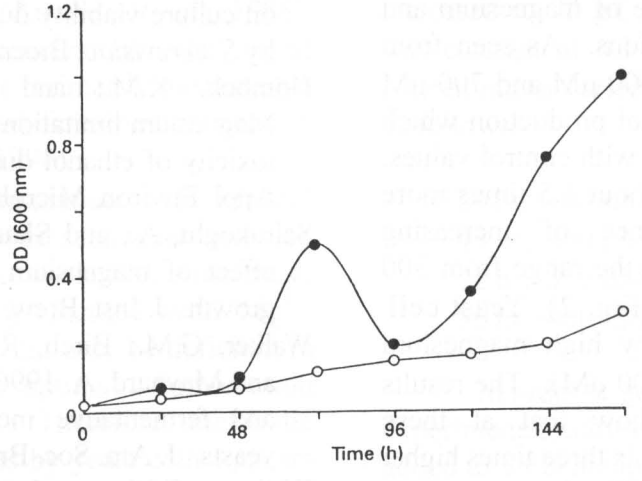

Fig. 3. The influence of Mg^{2+} concentrations from 100 to 700 μM on yeast growth.
Legend: o = control fermentation without added Mg^{2+}
● = fermentation in the presence of increasing amounts of Mg^{2+} from 100 to 700 μM.