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ANALYSIS OF MAGNESIUM INFLUENCE ON FERMENTATION WITH *Saccharomyces cerevisiae* YEASTS MIXED CULTURE

Larissa G. S. Silveira[1*],Paula R. F. Rosa [2] ,Fernanda F. Freitas[1] & Gabriel L. Castiglioni[3]

[1] *Chemical Engineering, Goiás Federal University/Chemical Institute, Goiânia, Brazil.*
[2] *Departure of Chemical Engineering, University of São Carlos, São Carlos, Brazil*
[3] *Chemical Engineering, Goiás Federal University/Agronomy School, Goiânia, Brazil*
** larissagssilveira@gmail.com*

ABSTRACT

The growth of fermentative microorganisms is closely related to the fermentative environment and consequently to the formation of the product. In this way, the addition of micro and macronutrients can increase the conversion of sugars to ethanol and promote the control of unwanted microorganisms. Although studies in area show a reduction in cell viability in presence of excess potassium, calcium and aluminum, other effects of these and other ions in fermentation medium are still unknown. Therefore, this study proposes an analysis of the role of magnesium in a fermentation with a mixed culture of *Saccharomyces cerevisiae* yeasts. The experiments were carried out with wild yeast and four industrial strains, for 72 hours, with 2.5 mL of synthetic must. The mineral option was defined after initial tests with eight minerals and the concentrations determined from literature data. Magnesium improved fermentation efficiency by up to 13% and reduced contamination from 24% to 12%. The results indicate that mineral is a viable alternative to promote ethanol production and minimize the negative effects of contamination.

Keywords: Synthetic wort. Wild yeast. Mineral supplementation. Metabolic activity.

1 INTRODUCTION

Microbial contamination has been a limiting factor to improve process performance from Brazilian ethanol production [1]. Prevention and control in bioethanol fermenters must be economical, general, easy to apply, environmentally friendly and non-toxic to yeast strains, humans, animals and plants [2].

Mineral nutrients facilitate yeast physiological processes and mechanisms associated to cell multiplication and growth, efficiency in sugars transformation, stress tolerance and flocculation control [3]. Furthermore, it is known that magnesium directly interferes in yeast development as an activator of extracellular enzymes [4].

All previously mentioned, this study investigates the effects of magnesium mineral supplementation on contaminating yeasts, based on fermentation of synthetic must and a mixed culture of Saccharomyces cerevisiae yeasts, to evaluate the contamination conditions in the process and consequently ethanol production.

2 MATERIAL & METHODS

The cultivated strains BG-1, CAT-1, FT-858L and PE-2 were available by Biochemical Engineering Laboratory of the Federal University of Goiás and the contaminating strain was isolated from a Brazilian ethanol producing plant [5]. To prepare the inoculum, each strain was removed from its respective maintenance plate and transferred to eppendorfs with 1mL liquid GPY medium (20 $g.L^{-1}$ of glucose, 5 $g.L^{-1}$ of yeast extract and 5 $g.L^{-1}$ of peptone). The tubes were shaken, incubated at 30ºC for 24h, and after, centrifuged at 8000 rpm for 2 minutes. The supernatant was removed, and the cells were resuspended in 1mL of sterile water to be added to the fermentation.

To ensure the reproducibility of the experiment, synthetic must was used with approximately 16% sugars (m/v) and following analytical standards for sugars, organic acids, amino acids, vitamins and mineral salts [6]. Some studies proposed concentrations to be supplemented in fermentations and were used as a theoretical reference to define the initial tests concentrations [7, 8, 9, 10]. The established values are in Table 1.

Table 1 Composition of initial tests with minerals and their respective concentrations in $g.L^{-1}$.

Initial test	1	2	3	4	5	6	7	8
Supplemented mineral	N	P	K	Ca	Mg	S	Cu	Fe
Concentration $g.L^{-1}$	5,000	0,400	2,000	0,300	1,000	0,400	0,015	0,100

The specifications for the fermentations were determined on the analysis of the incubation period. Contaminating strain is noxious at a proportion equal or greater than 30%. The tests were carried out with 2.5mL of synthetic must, the respective mineral concentration as highlighted in Table 1, 0.09 $g.L^{-1}$ of the contaminating yeast and 0.0525 $g.L^{-1}$ of each industrial yeast. , for 72h, at 30ºC and stirring at 150 rpm [5].

After the initial tests, tests were carried out supplementing only magnesium to evaluate the conduct of contaminating strain with mineral. Concentrations varied proportionally from 0.25 to 2.00 $g.L^{-1}$ and the specifications applied in the initial tests were maintained.

Sugars and other metabolites were analyzed by high-performance liquid chromatography (HPLC), removing 1mL from each test to centrifugation for 2 minutes at 8000 rpm. The supernatant was separated from the cells, diluted in a 1:5 ratio, filtered with Merck Millex-GP Filter filters with porosity 0.22 µm, injected into the ©Shimadzu brand chromatograph, model Prominence, with refractive index detectors (RID- 20 A) and UV-VIS (SPD-20A).

The fermentative efficiency (η) was calculated by residual sugars and ethanol produced as set out in equation 1.

$$\eta = \frac{Practical\ yield}{Theoretical\ yield} = \frac{Pf/(S0 - Sf)}{1-0{,}465} x100 = \frac{Pf/(S0 - Sf)}{0{,}535} x100 \quad (1)$$

3 RESULTS & DISCUSSION

Initially, triplicates of eight tests were carried out, supplemented respectively with nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, copper and iron. Each test was evaluated according to ethanol production, contamination level, sucrose consumed, cell concentration, glycerol production and residual glucose.

Using a machine learning algorithm, the data was related according to a regression problem and a score was calculated for the fermentation without supplementation called reference. Afterwards, a score was calculated to each test supplemented and compared with the reference as shown in Figure 1.

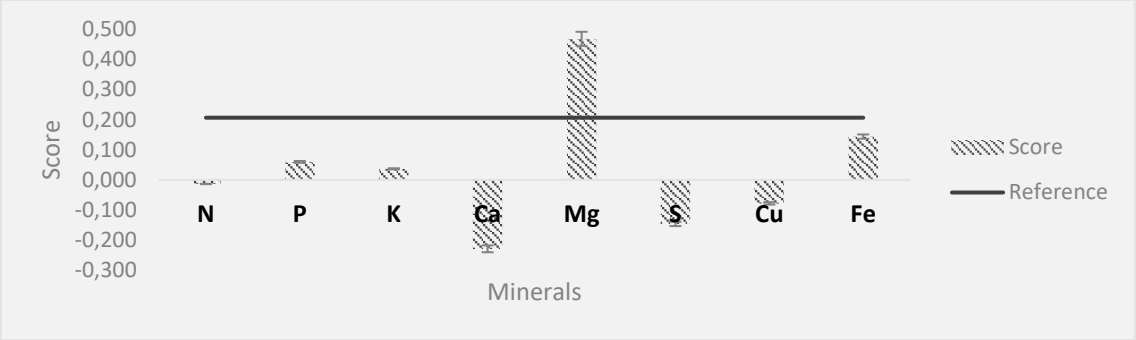

Figure 1 Evaluation of supplementation in the fermentative medium

Nitrogen, calcium, sulfur and iron showed negative scores, which suggests that results differ from reference fermentation and that increasing one parameter reduces other. The result infers that phosphorus, potassium and iron have a positive score but lower than reference. This may indicate that one or more parameters are negatively influenced by supplementation.

Only magnesium has a higher score than the control, indicating that the relationship between the parameters improves with the addition of the mineral. Magnesium interferes with yeast development by being an activator of extracellular enzymes. The element acts as a cofactor for more than 300 enzymes involved in metabolic and bioenergetic pathways [4,9].

In front of the favorable results, tests were carried out supplementing magnesium in concentrations ranging from 0.25 to 2.00$g.L^{-1}$. Literature data recommends that concentration of magnesium in alcoholic fermentation be between 0.07 and 0.20 $g.L^{-1}$ [7]. However, as the synthetic must used in analyzes was quantified at 0.05 $g.L^{-1}$ of magnesium, an extrapolation to the range was adopted to evaluate.

Triplicate assays were carried out and fermentations were compared with the reference as shown in Figure 2.

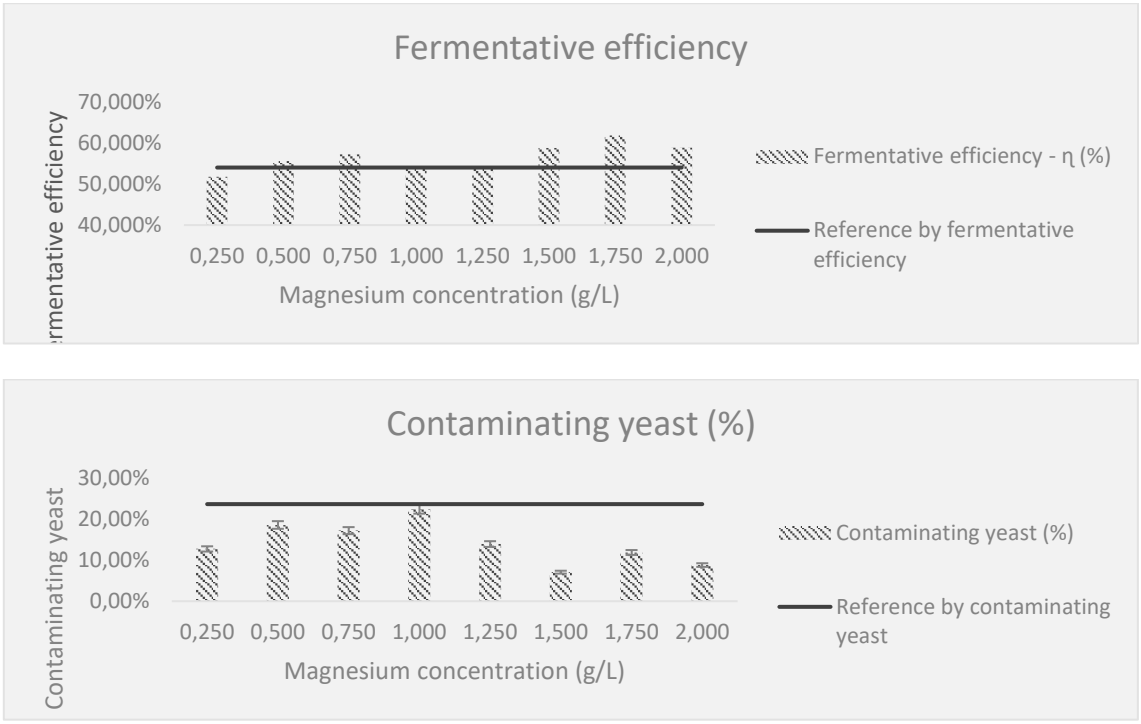

Figure 2 (A) fermentative efficiency and (B) contaminating yeast in fermentations supplemented with magnesium.

The results indicate that increasing the magnesium concentration in the fermentation medium, fermentation efficiency also increases. This result is similar to other studies that attribute the greater consumption of substrate to the specific transport system of yeasts that absorb magnesium intracellularly and encourage the consumption of sugars [10].

Furthermore, contamination was reduced at all concentrations evaluated, with a marked decrease when the medium received greater magnesium supplementation. The addition of magnesium favors rapid cell multiplication as well as growth of wild microorganisms such as various *E. coli* strains [11]. The experiments in present study demonstrated greater cell growth for industrial yeasts, however, the favoring of contaminating yeasts did not occur.

4 CONCLUSION

Magnesium supplementation is effective in optimizing alcoholic fermentations with mixed yeast cultures. In the tests carried out, the addition of the mineral promoted an increase in fermentative efficiency from 54% to 62% and reduced contamination by half. Furthermore, the presence of magnesium resulted in positive scenarios, with more ethanol produced and less contamination. In front the analyzes carried out, it can be stated that magnesium supplementation is a simple and economical alternative to promote an increase in ethanol production and minimize the presence of contaminating microorganisms.

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