Brazilian Journal of Food Technology

Original Article

Campinas, v. 19, e2015094, 2016
http://dx.doi.org/10.1590/1981-6723.9415
ISSN 1981-6723 on-line version

Magnesium capability to attenuate the toxicity of aluminum on the growth of *Saccharomyces cerevisiae* PE-2

A capacidade do magnésio em atenuar a toxicidade do alumínio no crescimento da levedura Saccharomyces cerevisiae PE-2

Samuel Mariano-da-Silva[1]*, Rafael Dal Bosco Ducatti[1], Ivan Pedro Murari[1], Fabio Pilon[1]

[1] *Universidade Federal da Fronteira Sul (UFFS), Department of Agronomy, Chapecó/SC - Brazil*

***Corresponding Author**

Samuel Mariano-da-Silva, Universidade Federal da Fronteira Sul (UFFS), Department of Agronomy, Caixa Postal: 181, CEP: 89802-112, Chapecó/SC - Brazil, e-mail: samuel.silva@uffs.edu.br

Cite as: *Magnesium capability to attenuate the toxicity of aluminum on the growth of Saccharomyces cerevisiae PE-2. Braz. J. Food Technol., v. 19, e2015094, 2016.*

Received: Nov. 23, 2015; Accepted: Nov. 07, 2016

Summary

The magnesium (Mg) capability to attenuate the toxicity of aluminum (Al) for the trehalose content, anaerobic growth, viability and budding rate of *Saccharomyces cerevisiae,* was studied in this work. Fermentations were carried out in triplicate with sterilized and diluted sugar cane media (4% total reducing sugars/pH 4.0) containing different Al (0.0, 50, 100 and 150 mg L^{-1}) and Mg (0.0, 50 and 100 mg L^{-1}) concentrations. The media were inoculated with 1 mL of 1% (wet basis) yeast suspension and incubated at 30°C, 70 rpm for 20 hours in orbital shaker. At specific times during fermentation portions of cell suspension were taken out and the biomass concentration, yeast viability, budding rate and trehalose content on cells determined. The increase of Al levels, from 0.0 up to 150 mg L^{-1}, showed a reduction on the yeast growth of approximately 95%, 55% and 18% as Mg increased from 0.0 to 50 and 100 mg L^{-1}, respectively. The trehalose content experienced its lowest reduction when greater amounts of Mg were added to the fermentation process. Cell viability showed greater reductions as the content of Al in the media increased. Magnesium effectively protected yeast cells against the deleterious effects of Al on cell growth, viability, budding and trehalose content.

Keywords: *Yeast viability; Fermentation; Trehalose content.*

Resumo

A capacidade do magnésio (Mg) em atenuar os efeitos tóxicos do alumínio (Al) para o conteúdo de trealose, o crescimento anaeróbio, a viabilidade e a taxa de brotamento em *Saccharomyces cerevisiae* foi estudada no presente trabalho. As fermentações foram realizadas em triplicatas, com suco de cana-de-açúcar esterilizado e diluído (4% de açúcares redutores totais/pH 4,0) contendo diferentes concentrações de Al (0,0, 50, 100 e 150 mg L^{-1}) e de Mg (0,0, 50 e 100 mg L^{-1}). Os meios foram inoculados com 1 mL de 1% (base úmida) de suspensão de levedura e incubados a 30°C, 70 rpm durante 20 horas, em agitador orbital. Em tempos específicos, durante a fermentação, porções da suspensão de células foram retiradas e a concentração de biomassa, a viabilidade das leveduras, a taxa de brotamento e o conteúdo de trealose foram determinados. O aumento dos teores de Al, de 0,0 a 150 mg L^{-1}, mostrou uma redução no crescimento da levedura de aproximadamente 95%, 55% e 18%, na presença de 0,0, 50 e 100 mg L^{-1} de Mg, respectivamente. O conteúdo de trealose sofreu a menor redução quando maiores teores de Mg foram adicionados ao meio de fermentação. A viabilidade celular apresentou maiores quedas à medida que se aumentou o conteúdo de Al no processo fermentativo. O magnésio protegeu eficazmente as células da levedura contra os efeitos deletérios do Al sobre o crescimento celular, a viabilidade, o brotamento e o teor de trealose.

Palavras-chave: *Viabilidade da levedura; Fermentação; Conteúdo de trealose.*

1 Introduction

It is crucial to consider the physiological conditions imposed by the industrial process on microorganisms present in the fermentation environment in order to identify the microbiological, physical and chemical elements that might be exerting, stimulating or stressing effects on these microorganisms (yeast and bacteria) (BASSO, 2006).

In the most widely fermentation processes used (Melle-Boinot), either in its fed batch version or in the continuous one, several factors which limit productivity, i.e., the uncontrolled microbiological contamination of the yeast due to biocides, the reduction in the yeast viability (BASSO et al., 2011; SOUZA, 2012) and the yeast low growth rate (WALKER, 2010), have already been diagnosed. Also, the high rates of aluminum (Al) in the must can be correlated to the decrease in the fermentative efficiency of yeasts (ANGELONI, 2009; FARIA, 2010; BASSO et al., 2011).

Despite being the most abundant metal in the Earth's crust, making up to 8% of the total weight of the Earth's external crust (ATSDR, 2008), Al ions can be very toxic to a variety of living organisms (DOREA; CLARKE, 2008). When in neutral or light acid (pH > 6.0) soils, Al is found in insoluble and harmless forms. Nevertheless, in more acid soils, the bioavailability and toxicity of Al are potentialized, as it is found in its ionic (hexahydrated or $Al(H_2O)_6^{+3}$) and/or cationic (Al^{+3}) forms (HOEKENGA, et al., 2003), which can be toxic to many plants, animals and microorganisms (DOREA; CLARKE, 2008; KIMOTO et al., 2010; SHAW; TOMLJENOVIC, 2013).

Aluminum toxicity is especially raised when soil acidity is intensified by land-use intensification, i.e., industrial activities, and fertilization with acid action fertilizers (DIDHAM et al., 2015; FAGERIA; NASCENTE, 2014). Tropical South America has an amount of 85% of acid soils, of which about 24% in located in the central part of Brazil (FAGERIA; NASCENTE, 2014). Liming the soils to correct acidity is a good strategy to enhance agricultural productivity (PAGANI; MALLARINO, 2012), however, the high amount of limestone necessary for soil correction might not be a favorable economical choice, resulting in applications of lime below what is necessary (GOULDING, 2016).

When sugarcane is cultivated in low pH soils, consequently containing high amounts of available Al, it is expected that the juice used to prepare the must would contain a significant amount of the element (BASSO et al., 2011), transferring it to the industrial process of carburant alcohol production. Another possibility for the entrance of Al in the industrial process is the use of water treated with potassium alum or aluminum sulfate, a practice, which is used in many industrial facilities (ARANHA, 2002).

Many studies have demonstrated (ANGELONI, 2009; BASSO, 2006; BASSO et al., 2011) that industrial must used in many distilleries in São Paulo State might present Al rates which rank from 8 to 40 mg L^{-1}, reaching in some cases amounts as high as 500 mg L^{-1}, which are normally associated to the decrease in the yeast cell viability. Aranha (2002) reports analyses carried out by Copersucar Centro Tecnológico that have shown Al average rates of 156 mg L^{-1} in the juice destined to fermentation, although, Oliveira et al. (2009) assert that concentrations above 13.5 mg L^{-1} already exert depressive action over fermentation.

In the industrial process of ethanol production yeast are reused from one fermentative cycle to another, in 6-10-hour fermentation, performing easily 2 fermentative cycles a day throughout a harvest that lasts from 200 to 250 days. These yeast intense cycles – which are a specific feature of the process settled in the country – could lead to an accumulative effect of Al, generating toxic effects of the metal itself in lower levels than those presented in the specific literature (ANGELONI, 2009; FARIA, 2010).

Interestingly, it is noticeable that, although Al toxicity is widely reported in biotechnological processes such as bakery (JALBANI et al., 2007), vinification (GALANI-NIKOLAKAKI; KALLITHRAKAS-KONTOS, 2007), biomass production (REHMUS et al., 2014), brewery (SCHWALFENBERG et al., 2013) and carburant alcohol production (BASSO et al., 2011), only a few studies have been published about it in physiological conditions of a biotechnological process. Therefore, the aim of this study was to assess the effects of Al toxicity on the trehalose content, anaerobic growth, viability and budding rate of *S. cerevisiae* and the capability of Mg to attenuate this toxicity.

2 Material and methods

2.1 Preparation of lab equipment

All reusable lab equipment (glass, quartz, polyethylene, Polytetrafluoroethylene, etc) were prepared for use by washing with detergent, rinsing with ultra pure water and soaking them for four hours in a mixture of nitric acid, hydrochloric acid and water (1:2:9) followed by another rinsing with ultra pure water and heat drying (MARIANO-DA-SILVA et al., 2009).

2.2 Yeast

Saccharomyces cerevisiae PE-2 was kindly provided by the Latino Americana Company (LNF).

2.3 Yeast pre-growth

Yeast was reactivated in YEPD (Yeast Extract Peptone Dextrose) medium from a pure-culture (lyophilized) and pre-grown, at 30 °C, in sterilized (autoclaved at 1 ATM, 120°C for 20 minutes) molasses medium with 6% TRS (total reducing

sugars), supplemented with KH_2PO_4 (8.36 mmol L^{-1}), $(NH_4)_2SO_4$ (5 mmol L^{-1}), urea (38.75 mmol L^{-1}), $MgSO_4.H_2O$ (3.57 mmol L^{-1}), $ZnSO_4.7H_2O$ (0.10 mmol L^{-1}), $MnSO_4.H_2O$ (0.12 mmol L^{-1}) and linolenic acid (0.11 mmol L^{-1}). Cells from the late-exponential growth phase were harvested by centrifugation (800 G, 20 min) and re-suspended in distilled deionized water to a final concentration of 1g (fresh weight) 100 mL^{-1} (MARIANO-DA-SILVA; BASSO, 2004).

2.4 Growth assay

Fermentation was carried out in triplicate with sterilized (autoclaved at 1 ATM, 120ºC for 20 minutes) 75 mL of diluted sugar cane (2% total reducing sugars/pH 4.0) medium in 125 mL Erlenmeyer flasks sealed with anhydrous cotton and aluminum foil. The flasks received different Al (0.0, 50, 100 and 150 mg L^{-1}) and magnesium (Mg) concentrations (0.0, 50 and 100 mg L^{-1}), for a total of 12 treatments. The flasks were inoculated in aseptic conditions with 1 mL of 1% (wet basis) yeast suspension and incubated at 30 ºC, 70 rpm for 20 hours in orbital shaker (MARIANO-DA-SILVA et al., 2009).

2.5 Growth curves

At specific times during fermentation (0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 hours), 1 mL portions of cell suspension were removed and transferred to a test-tube with 9 mL of deionized water. The biomass concentration was determined by turbidity measurements at 570 nm (Bausch and Lomb) using a standard-line previously performed.

2.6 Cell counting

At specific times during fermentation (0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 hours) 0.5 mL of each cell suspension was sampled, diluted, erythrosine colored and directly counted with a microscope for yeast viability and budding rate evaluation, according to Amorin et al. (1989).

2.7 Yeast trehalose

During the growth (every 2 hours), trehalose was extracted from 60 mg of washed cells (fresh weight) using 2 mL of 0.5 mol L^{-1} trichloroacetic acid kept in ice bath for 20 minutes (the suspension was frequently shaken). The suspension was then centrifuged (TREVELYAN; HARRISON, 1956a, b) and 0.2 mL of each supernatant was subjected to anthrone reaction according to Brin (1966).

2.8 Statistical analysis

The response of all the variables was analyzed using JMP Pro 12® (SAS Institute, Cary, NC). The variables were submitted to F-tests (ANOVA) following casual delineation in crossed model with triplicates. The averages were compared using Tukey's HSD multiple comparison method (alpha = 0.01) (ARES; GRANATO, 2014).

3 Results and discussion

Figure 1 shows the simultaneous effects of Al and Mg on the growth of *Saccharomyces cerevisiae*. In the absence of Mg (Figure 1A) there was a decrease in the growth rate of the yeast. Moreover, in the concentrations of 50 and 100 mg L^{-1} a delay at the end of the Log phase was observed, which had already been reported by, Mariano-da-Silva and Basso (2004) and Oliveira et al. (2012) concerning cadmium.

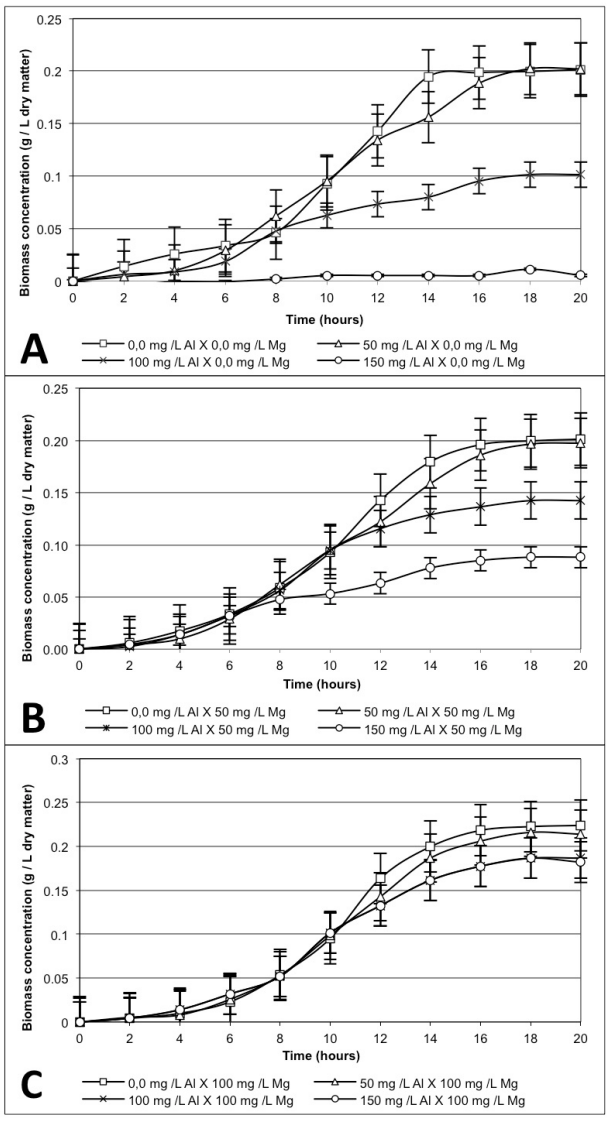

Figure 1. Growth curves of *Saccharomyces cerevisiae*: (A) in the presence of different Al concentrations and absence of Mg; (B) in the presence of different Al concentrations and 50 mg L^{-1} of Mg; (C) in the presence of different Al concentrations and 100 mg L^{-1} of Mg.

Oliveira et al. (2009) obtained, in their study, a higher depressive effect on the biomass concentration of *S. cerevisiae* when they used 54 mg L^{-1} of Al in the medium, which resulted in a reduction of approximately 19% when compared to the control (0.0 mg L^{-1}). For the specific growth rate of *S. cerevisiae* these authors concluded that increments in the Al concentration from 13.5 to 54 mg L^{-1} reduced the growth rate from 14% to 56%, in media containing 0.0 and 150 g L^{-1} of vinasse content, respectively. In our work we found a reduction of biomass concentration, for our treatment "50 mg L^{-1} Al X 0.0 mg L^{-1} Mg". This higher toxicity, found by Oliveira et al. (2009), might be due to the growth medium used by the authors (YED – yeast dextrose medium), which is less complex than the sugarcane juice medium and, thus, has fewer potentially chelating/sequestrating agents (MARIANO-DA-SILVA et al., 2009).

When 100 and 150 mg L^{-1} of Al were added, a decrease in the growth rate was observed and the final cell density was reduced by approximately 50 and 100%, respectively, in comparison to the control (0 mg L^{-1}).

In the absence of Al, an addition of 100 mg L^{-1} of Mg caused the prolongation of the exponential phase from 14 to 18 hours, leading to a higher accumulation of cell mass (Figures 1B and 1C), probably due to the increase in the carbohydrate conversion and the decrease of time required for this conversion (DOMBEK; INGRAM, 1986; WALKER et al., 2003).

Aranha (2002) asseverates that the action of Al, although discreet, is characterized by the reduction of biomass production. According to the author, cell viability is reduced possibly due to the reduction of trehalose and glycogen levels in the yeast cells.

Aluminum toxicity significantly decreases with Mg increase, following the findings of Trofimova et al. (2010). In the media containing 50 mg L^{-1} of Mg, Al toxicity was slightly attenuated (Figure 1B), however, increasing the Mg concentration to 100 mg L^{-1} suppressed the toxicity of Al in all the concentrations tested.

Aluminum has been shown to cause toxicity in *S. cerevisiae* through several mechanisms, as being an inhibitor of hexokinases (WOMACK; COLOWICK, 1979), glucose-6-phosphate dehydrogenase (CHO; JOSHI, 1989) and isocitric dehydrogenase (YOSHINO et al., 1992). The hypothesis that the Al toxicity mechanism was due to its bonds to catalytic sites of enzymes dependent on metal-activation was initially raised in our study. However, Jones and Kochian (1997) demonstrated the high affinity of the Al ion for phosphatidylinositol-4,5-bisphosphate, component of the signaling transduction membrane, showing that the cause of Al toxicity is not due to the enzymes interaction/inhibition, but changes in the membrane permeability instead, which according to Li et al. (2011) can cause an intracellular calcium (Ca) homeostasis affecting the apoptosis in yeast.

MacDiarmid and Gardner (1998) and Schott and Gardner (1997) disclosed that it is the super-expression of the Mg transport systems in *S. cerevisiae* that confer tolerance to the Al ion and that Al toxicity would be the consequence of a reduction in the Mg influx by such carrier. Trofimova et al. (2010) show that Mg play an important role on yeast growth and metabolism, i.e., the function of key enzymes and cell membrane stabilization.

Tables 1 and 2 show the cell viability and budding rate at different Al and Mg concentrations, respectively.

In treatments without Al, or when Mg was added, the most significant effect for both parameters was the decrease of cell viability and the increment of the budding rate over time. In treatments without Mg and with 50, 100 and 150 mg L^{-1} of Al we found a strong decrease in the yeast cell viability and budding when compared to the control. Adding 100 mg L^{-1} of Mg resulted in a considerable loss of viability and restored the budding rates. These parameters (cell viability and budding rate) reflect the physiological state of the culture, provided that, cell viability indicates the physiological stress to which the yeast was submitted, and the budding rate offers information on the development of the culture (MARIANO-DA-SILVA, 1998; RODRÍGUES-PORRATA et al., 2008; OLIVEIRA et al., 2012).

The pH decreased during growth reaching a level of 2.98 at the stationary phase (Table 3), going into accordance to the findings of MacDiarmid and Gardener (1996). When working with the YPD medium, the authors observed that the pH decreased during growth, from 3.5 to 2.9. The fermentative growth of yeast is known to reduce the medium pH (WALKER, 2010) by extrusion of metabolites.

It was possible to observe that 100 to 150 mg L^{-1} of Al reduced the rate of CO_2 production (Figure 2A). Nonetheless, when 50 and 100 mg L^{-1} of Mg were added, the deleterious action of Al was suppressed (Figure 2B and 2C). The highest production of CO_2 was found in the treatment containing 100 mg L^{-1} of Mg (Figure 2C). That might be due to the fact that Mg is an important enzymatic cofactor of several glycolytic enzymes, stimulating glycolysis and the consequent CO_2 production (BIRCH et al., 2003; LIM et al., 2011).

Intracellular trehalose content decreased during fermentation in all the treatments (Figure 3); however, this decrease was greater for the treatments with 50, 100 and 150 mg L^{-1} of Al. When adding Mg to the media, we observed an attenuation of the toxic effects of Al on the trehalose content of cells. Moreover, for the treatments "0,0 mg L^{-1} Al x 100 mg L^{-1} Mg" and "50 mg L^{-1} Al x 100 mg L^{-1} Mg" the final trehalose contents

Table 1. Cell viability (%) during fermentation using media with different Al and Mg concentrations.

Treatments	Fermentation time (h)										
	0	2	4	6	8	10	12	14	16	18	20
0.0 mg L^{-1} Al X 0.0 mg L^{-1} Mg	93.66^{Aa}	90.00^{Aa}	89.74^{Aa}	89.19^{Aa}	87.78^{Aa}	88.46^{Aa}	88.36^{Aa}	87.75^{Aa}	87.55^{Aa}	88.07^{Aa}	76.63^{Bb}
50 mg L^{-1} Al X 0.0 mg L^{-1} Mg	93.66^{Aa}	78.13^{Bb}	77.78^{Bb}	69.51^{Cb}	71.11^{Cb}	71.31^{Cb}	68.10^{Cb}	67.18^{Cb}	68.95^{Cb}	67.23^{CDb}	67.61^{Cb}
100 mg L^{-1} Al X 0.0 mg L^{-1} Mg	93.66^{Aa}	68.75^{Cb}	68.29^{Cb}	69.12^{Cb}	66.98^{Db}	67.20^{Cb}	64.67^{Cb}	65.02^{Cb}	62.45^{Cb}	60.33^{Eb}	60.06^{Db}
150 mg L^{-1} Al X 0.0 mg L^{-1} Mg	93.66^{Aa}	70.00^{Cb}	61.11^{Cb}	59.44^{Db}	46.96^{Ec}	47.92^{Dc}	46.53^{Dc}	43.59^{Dc}	40.00^{Dc}	38.89^{Fc}	37.50^{Ec}
0.0 mg L^{-1} Al X 50 mg L^{-1} Mg	93.66^{Aa}	94.44^{Aa}	87.56^{Aa}	88.81^{Aa}	89.24^{Aa}	88.97^{Aa}	88.48^{Aa}	87.23^{Aa}	88.28^{Aa}	86.29^{Aa}	87.12^{Aa}
50 mg L^{-1} Al X 50 mg L^{-1} Mg	93.66^{Aa}	80.95^{Bb}	80.49^{Bb}	77.22^{Bb}	75.82^{BCb}	76.19^{Bb}	77.31^{Bb}	76.28^{Bb}	69.86^{Cb}	71.43^{Cb}	67.03^{Cb}
100 mg L^{-1} Al X 50 mg L^{-1} Mg	93.66^{Aa}	78.77^{Bb}	77.61^{Bb}	75.26^{Bb}	74.90^{BCb}	73.00^{Cb}	72.76^{Cb}	73.82^{Bb}	69.33^{Cb}	68.94^{CDb}	67.07^{Cb}
150 mg L^{-1} Al X 50 mg L^{-1} Mg	93.66^{Aa}	78.13^{Bb}	77.78^{Bb}	69.14^{Cb}	70.10^{Cb}	69.26^{Cb}	69.16^{Cb}	68.55^{Cb}	69.31^{Cb}	65.98^{Db}	65.62^{Cb}
0.0 mg L^{-1} Al X 100 mg L^{-1} Mg	93.66^{Aa}	93.80^{Aa}	90.59^{Aa}	92.17^{Aa}	90.08^{Aa}	87.06^{Aa}	86.88^{Aa}	86.10^{Aa}	88.45^{Aa}	86.35^{Aa}	87.55^{Aa}
50 mg L^{-1} Al X 100 mg L^{-1} Mg	93.66^{Aa}	84.19^{Bb}	82.98^{Bb}	82.11^{Bb}	81.05^{Bb}	80.67^{Bb}	80.00^{Bb}	80.33^{Bb}	80.38^{Bb}	79.20^{Bb}	78.13^{Bb}
100 mg L^{-1} Al X 100 mg L^{-1} Mg	93.66^{Aa}	83.98^{Bb}	82.61^{Bb}	81.82^{Bb}	79.67^{Bb}	80.00^{Bb}	80.42^{Bb}	79.20^{Bb}	78.26^{Bb}	79.06^{Bb}	76.52^{Bb}
150 mg L^{-1} Al X 100 mg L^{-1} Mg	93.66^{Aa}	81.86^{Bb}	82.98^{Bb}	80.80^{Bb}	81.05^{Bb}	78.13^{Bb}	76.74^{Bb}	75.35^{Bb}	75.74^{Bb}	76.80^{Bb}	77.30^{Bb}
Standard deviation: 8.21%											

The averages followed by the same letters (capital on the same column or small on the same line) did not differ from each other, according to Tukey's HSD tests at a confidence level of 1%.

Table 2. Budding rate (%) during fermentation using media with different Al and Mg concentrations.

Treatments	Fermentation time (h)										
	0	2	4	6	8	10	12	14	16	18	20
0.0 mg L^{-1} Al X 0.0 mg L^{-1} Mg	14.2^{Aab}	9.8^{Ab}	10.2^{Ab}	10.8^{Ab}	12.9^{Ab}	16.0^{Aa}	17.1^{Aa}	17.8^{Aa}	18.1^{Aa}	15.3^{Aab}	15.1^{Aab}
50 mg L^{-1} Al X 0.0 mg L^{-1} Mg	14.2^{Aa}	9.7^{Ab}	10.0^{Ab}	9.6^{Ab}	9.6^{Ab}	10.0^{Bb}	11.0^{Bb}	10.4^{Bb}	10.5^{Bb}	9.0^{Bb}	8.9^{Bb}
100 mg L^{-1} Al X 0.0 mg L^{-1} Mg	14.2^{Aa}	9.9^{Ab}	7.1^{ABb}	4.2^{Bc}	1.9^{Bd}	0.5^{Cd}	0.7^{Cd}	0.3^{Cd}	0.5^{Cd}	0.6^{Cd}	0.2^{Cd}
150 mg L^{-1} Al X 0.0 mg L^{-1} Mg	14.2^{Aa}	8.7^{Ab}	4.3^{Bc}	0.0^{Cd}	0.0^{Bd}	0.0^{Cd}	0.0^{Cd}	0.0^{Cd}	0.0^{Cd}	0.0^{Cd}	0.0^{Cd}
0.0 mg L^{-1} Al X 50 mg L^{-1} Mg	14.2^{Ab}	10.0^{Ac}	11.2^{Ac}	11.9^{Ac}	12.8^{Ac}	15.7^{Aab}	17.5^{Aa}	18.0^{Aa}	18.3^{Aa}	17.4^{Aa}	15.3^{Aab}
50 mg L^{-1} Al X 50 mg L^{-1} Mg	14.2^{Aa}	8.6^{Ab}	8.8^{Ab}	8.5^{Ab}	7.9^{Ab}	7.9^{Bb}	7.9^{Bb}	7.8^{Bb}	6.9^{Bb}	6.9^{Bb}	6.7^{Bb}
100 mg L^{-1} Al X 50 mg L^{-1} Mg	14.2^{Aa}	9.0^{Ab}	8.2^{Ab}	8.1^{Ab}	7.2^{Ab}	7.8^{Bb}	7.5^{Bb}	7.1^{Bb}	6.9^{Bb}	6.3^{Bb}	6.1^{Bb}
150 mg L^{-1} Al X 50 mg L^{-1} Mg	14.2^{Aa}	9.4^{Ab}	7.0^{ABc}	6.9^{ABc}	6.4^{Ac}	6.7^{Bc}	5.9^{BCcd}	6.0^{Bcd}	6.3^{Bc}	5.9^{BCcd}	5.5^{Bd}
0.0 mg L^{-1} Al X 100 mg L^{-1} Mg	14.2^{Aab}	11.6^{Ab}	11.7^{Ab}	12.9^{Aab}	13.7^{Aab}	15.4^{Aab}	17.6^{Aa}	17.5^{Aa}	18.6^{Aa}	17.5^{Aa}	16.9^{Aa}
50 mg L^{-1} Al X 100 mg L^{-1} Mg	14.2^{Aab}	9.2^{Ab}	9.8^{Ab}	9.8^{Ab}	10.9^{A}	14.9^{Aab}	15.2^{Aab}	15.8^{Aab}	18.1^{Aa}	17.7^{Aa}	17.5^{Aa}
100 mg L^{-1} Al X 100 mg L^{-1} Mg	14.2^{Aa}	8.9^{Ab}	8.8^{Ab}	9.5^{Ab}	9.9^{Ab}	9.8^{Bb}	9.2^{Bb}	9.8^{Bb}	9.4^{Bb}	9.0^{Bb}	9.7^{Bb}
150 mg L^{-1} Al X 100 mg L^{-1} Mg	14.2^{Aa}	9.0^{Ab}	8.2^{Abc}	8.9^{Ab}	8.2^{Abc}	8.0^{Bbc}	8.1^{Bbc}	7.4^{Bbc}	7.9^{Bbc}	6.7^{Bc}	6.5^{Bc}
Standard deviation: 16.21%											

The averages followed by the same letters (capital on the same column or small on the same line) did not differ from each other, according to Tukey's HSD tests at a confidence level of 1%.

Table 3. pH during fermentation using media with different Al and Mg concentrations.

Treatments	Fermentation time (h)										
	0	2	4	6	8	10	12	14	16	18	20
0.0 mg L^{-1} Al X 0.0 mg L^{-1} Mg	5.00^{Aa}	4.92^{Aa}	4.67^{Aa}	4.38^{Aab}	4.10^{Ab}	3.89^{Ab}	3.51^{Ab}	3.26^{Abc}	3.20^{Ac}	3.16^{Ac}	3.14^{Ac}
50 mg L^{-1} Al X 0.0 mg L^{-1} Mg	5.00^{Aa}	4.90^{Aa}	4.56^{Aa}	4.25^{Aab}	4.00^{Ab}	3.70^{Ab}	3.47^{Ab}	3.20^{Ac}	3.13^{A}	3.04^{Ac}	2.99^{Ac}
100 mg L^{-1} Al X 0.0 mg L^{-1} Mg	5.00^{Aa}	4.88^{Aa}	4.57^{Aa}	4.23^{Aab}	4.01^{Ab}	3.75^{Ab}	3.43^{Ab}	3.21^{Ac}	3.12^{A}	3.05^{Ac}	2.98^{Ac}
150 mg L^{-1} Al X 0.0 mg L^{-1} Mg	5.00^{Aa}	4.90^{Aa}	4.60^{Aa}	4.49^{Aab}	4.31^{Aab}	4.17^{Ab}	3.90^{Ab}	3.77^{Abc}	3.55^{Abc}	3.28^{Ac}	3.12^{Ac}
0.0 mg L^{-1} Al X 50 mg L^{-1} Mg	5.00^{Aa}	4.92^{Aa}	4.67^{Aa}	4.50^{Aa}	4.29^{Aab}	4.15^{Ab}	3.89^{Ab}	3.69^{Abc}	3.53^{Abc}	3.30^{Ac}	3.11^{Ac}
50 mg L^{-1} Al X 50 mg L^{-1} Mg	5.00^{Aa}	4.88^{Aa}	4.66^{Aa}	4.49^{Aa}	4.30^{Aab}	4.12^{Ab}	3.90^{Ab}	3.67^{Abc}	3.55^{A}	3.28^{A}	3.14^{Ac}
100 mg L^{-1} Al X 50 mg L^{-1} Mg	5.00^{Aa}	4.93^{Aa}	4.60^{Aa}	4.37^{Aab}	4.05^{Ab}	3.77^{Ab}	3.44^{Ab}	3.29^{Ab}	3.15^{A}	3.09^{Ac}	3.00^{Ac}
150 mg L^{-1} Al X 50 mg L^{-1} Mg	5.00^{Aa}	4.86^{Aa}	4.59^{Aa}	4.28^{Aab}	4.00^{Ab}	3.79^{Ab}	3.49^{Ab}	3.24^{Ac}	3.14^{A}	3.09^{Ac}	2.98^{Ac}
0.0 mg L^{-1} Al X 100 mg L^{-1} Mg	5.00^{Aa}	4.90^{Aa}	4.63^{Aa}	4.42^{Aab}	4.34^{Aab}	3.80^{Ab}	3.67^{Ab}	3.59^{Abc}	3.37^{Abc}	3.29^{Ac}	3.10^{Ac}
50 mg L^{-1} Al X 100 mg L^{-1} Mg	5.00^{Aa}	4.88^{Aa}	4.65^{Aa}	4.46^{Aab}	4.35^{Aab}	3.93^{Ab}	3.70^{Ab}	3.55^{Abc}	3.42^{Abc}	3.30^{Ac}	3.09^{Ac}
100 mg L^{-1} Al X 100 mg L^{-1} Mg	5.00^{Aa}	4.90^{Aa}	4.66^{Aa}	4.44^{Aab}	4.35^{Aab}	4.20^{Aab}	3.93^{Ab}	3.74^{Abc}	3.55^{Abc}	3.23^{Ac}	3.12^{Ac}
150 mg L^{-1} Al X 100 mg L^{-1} Mg	5.00^{Aa}	4.91^{A}	4.59^{Aa}	4.45^{Aab}	4.33^{Aab}	4.21^{Aab}	3.92^{Ab}	3.75^{Abc}	3.54^{Abc}	3.22^{Ac}	3.13^{Ac}
Standard deviation: 14.89%											

The averages followed by the same letters (capital on the same column or small on the same line) did not differ from each other, according to Tukey's HSD tests at a confidence level of 1%.

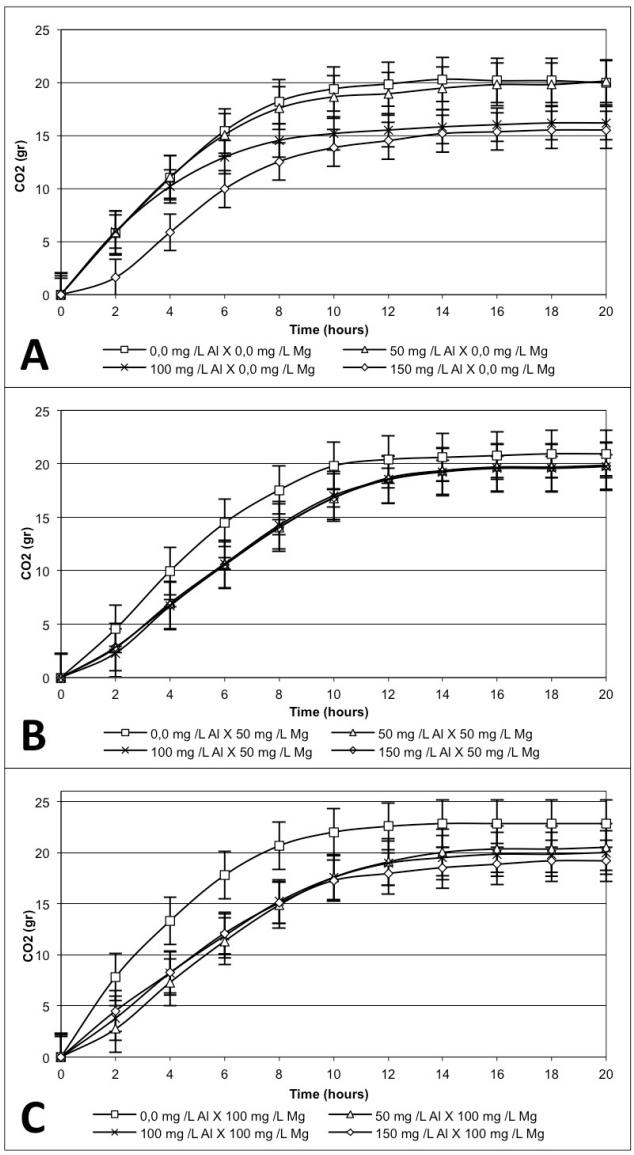

Figure 2. CO_2 produced by *Saccharomyces cerevisiae*: (A) in the presence of different Al concentrations and absence of Mg; (B) in the presence of different Al concentrations and 50 mg L^{-1} of Mg; (C) in the presence of different Al concentrations and 100 mg L^{-1} of Mg.

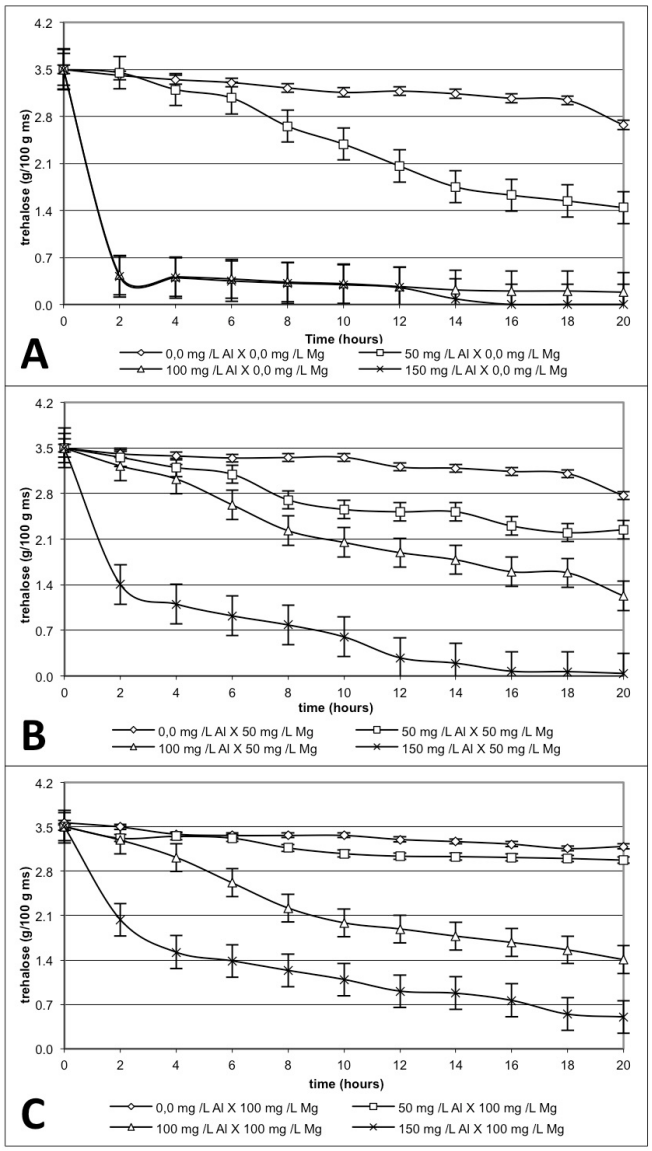

Figure 3. Trehalose content in: (A) presence of different Al concentrations and absence of Mg; (B) presence of different Al concentrations and 50 mg L^{-1} of Mg; (C) presence of different Al concentrations and 100 mg L^{-1} of Mg.

were higher than those found in the control. According to Basso et al. (2011), aluminum is, in toxic levels, responsible for decreasing fermentation performance, negatively affecting the yeast viability, cellular trehalose levels, fermentation rate and ethanol yield, on the other hand, Mg can alleviate this toxicity by conforming the cells higher resistance (TROFIMOVA et al., 2010; LIM et al., 2011).

Intracellular trehalose contents reflect the stress to which cells are submitted, keeping an optimal relationship with the growth and the viability rate (MARIANO-DA-SILVA et al., 2007, 2009; OLIVEIRA et al., 2012). It is possible that Al, likewise cadmium (MARIANO-DA-SILVA, 1998; OLIVEIRA et al., 2012), inhibited the transport of glucose in the cell (new studies to prove this hypothesis are needed), thus, reducing the intracellular contents of trehalose in cells.

4 Conclusions

The increase of Al levels in the media caused a significant reduction of the yeast growth, trehalose content and cell viability, however, magnesium effectively protected yeast cells against the toxic effects of Al. These results have shown that the use of Mg in the fermentation media may be a good option in order to obtaining better results concerning faster fermentative cycles, longer yeast cell life, and greater resistance to toxic metals present during the fermentation process.

References

AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY – ATSDR. **Toxicological profile for aluminum**. Atlanta, 2008. Available at: <http://www.atsdr.cdc.gov/toxprofiles/tp22.pdf>. Accessed on: 02 aug. 2016.

AMORIN, H. V.; OLIVEIRA, A. J.; ZAGO, E. A.; BASSO, L. C.; GALLO, C. R. **Processos de fermentação alcoólica, seu controle e monitoramento**. Piracicaba: FERMENTEC, 1989. p. 146.

ANGELONI, L. H. P. **Bioacúmulo de alumino e seus efeitos tóxicos na fermentação alcoólica em linhagens industriais de Saccharomyces cerevisiae**. 2009. 124 f. Dissertação (Mestrado em Microbiologia Agrícola)-Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2009.

ARANHA, D. A. D. **Efeitos do alumínio sobre a fermentação alcoólica**. 2002. 86 f. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos)-Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2002.

ARES, G.; GRANATO, D. **Mathematical and statistical methods in food science and technology**. Nova Jersey: John Wiley & Sons Inc, 2014. p. 536.

BASSO, L. C. Fisiologia e ecologia microbiana. In: WORKSHOP TECNOLÓGICO SOBRE PRODUÇÃO DE ETANOL, 1., 2006, Lorena. **Anais...** Lorena: Escola de Engenharia de Lorena, 2006. p. 7. Available at: <http://www.apta.sp.gov.br/cana/anexos/Paper_sessao_2_ Basso.pdf>. Accessed on: 16 july 2015.

BASSO, L. C.; BASSO, T. O.; ROCHA, S. N. Ethanol production in Brazil: the industrial process and its impact on yeast fermentation. In: BERNARDES, M. A. S. **Biofuel production**: recent developments and prospects. Rijeka: InTech, 2011. chap. 5, p. 85-100.

BIRCH, R. M.; CIANI, M.; WALKER, G. M. Magnesium, calcium and fermentative metabolism in wine yeast. **Journal of Wine Research**, London, v. 14, n. 1, p. 3-15, 2003. http://dx.doi.org/10.1080/0957126032000114973.

BRIN, M. Tranketalose: clinical aspects. In: COLOWICK, S. P.; KAPLAN, N. O. (Ed.). **Methods in enzymology**. New York: Academic Press, 1966. chap. 9, p. 506-514.

CHO, S. W.; JOSHI, J. G. Inactivation of baker's – yeast glucose-6-phosphate-dehidrogenase by aluminum. **Biochemistry**, Washington, v. 28, n. 8, p. 3613-3618, 1989. PMid:2663074. http://dx.doi.org/10.1021/bi00434a069.

DIDHAM, R. K.; BARKER, G. M.; BARTLAM, S.; DEAKIN, E. L.; DENMEAD, L. H.; FISK, L. M.; PETERS, J. M.; TYLIANAKIS, J. M.; WRIGHT, H. R.; SCHIPPER, L. A. Agricultural intensification exacerbates spillover on soil biogeochemistry in adjacent forest remnants. **PLoS One**, San Francisco, v. 10, n. 1, p. e0116474, 2015. PMid:25575017. http://dx.doi.org/10.1371/journal.pone.0116474.

DOMBEK, K. M.; INGRAM, L. O. Magnesium limitation and its role in the apparent toxicity of ethanol in *Saccharomyces cerevisiae* fermentation. **Applied and Environmental Microbiology**, Washington, v. 51, n. 1, p. 197-200, 1986. PMid:3513699.

DOREA, C. C.; CLARKE, B. A. Effect of aluminum on microbial respiration. **Water, Air, and Soil Pollution**, Guildford, v. 189, n. 1, p. 353-358, 2008. http://dx.doi.org/10.1007/s11270-007-9553-3.

FAGERIA, N. K.; NASCENTE, A. S. Management of soil acidity of South American soils for sustainable crop production. **Advances in Agronomy**, San Diego, v. 128, p. 221-275, 2014. http://dx.doi.org/10.1016/B978-0-12-802139-2.00006-8.

FARIA, F. P. **Alumínio**: ação tóxica sobre a levedura *Saccharomyces cerevisiae* PE-2 e o efeito protetor do ácido cítrico e magnésio. 2010. 78 f. Dissertação (Mestrado em Agronomia)-Universidade Federal de Goiás, Jataí, 2010.

GALANI-NIKOLAKAKI, S. M.; KALLITHRAKAS-KONTOS, N. G. Elemental content of wines. In: SZEFER, P.; NRIAGU, J. O. **Mineral components in foods**. Boca Raton: CRC Press, 2007. chap. 8, p. 323-338. http://dx.doi.org/10.1201/9781420003987.ch8.

GOULDING, K. W. T. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. **Soil Use and Management**, Wallingford, v. 32, n. 3, p. 390-399, 2016. PMid:27708478. http://dx.doi.org/10.1111/sum.12270.

HOEKENGA, O. A.; VISION, T. J.; SHAFF, J. E.; MONFORTE, A. J.; LEE, G. P.; HOWELL, S. H.; KOCHIAN, L. V. Identification and characterization of aluminum tolerance loci in Arabidopsis (Landsberg erecta x Columbia) by quantitative trait locus mapping. A physiologically simple but genetically complex trait. **Plant Physiology**, Lancaster, v. 132, n. 2, p. 936-948, 2003. PMid:12805622. http://dx.doi.org/10.1104/pp.103.023085.

JALBANI, N.; KAZI, T. G.; JAMALI, M. K.; ARAIN, B. M.; AFRIDI, H. L.; BALOCH, A. Evaluation of aluminum contents in different bakery foods by electrothermal atomic absorption spectrometer. **Journal of Food Composition and Analysis**, San Diego, v. 20, n. 3-4, p. 226-231, 2007. http://dx.doi.org/10.1016/j.jfca.2006.10.004.

JONES, D. L.; KOCHIAN, L. V. Aluminum interaction with plasma membrane lipids and enzyme metal binding sites and its potential role in Al cytotoxicity. **FEBS Letters**, Amsterdam, v. 400, n. 1, p. 51-57, 1997. PMid:9000512. http://dx.doi.org/10.1016/S0014-5793(96)01319-1.

KIMOTO, K.; AIZAWA, T.; URAI, M.; VE, N. B.; SUZUKI, K.; NAKAJIMA, M.; SUNAIRI, M. *Acidocella aluminiidurans* sp nov., an aluminum-tolerant bacterium isolated from *Panicum repens* grown in a highly acidic swamp in actual acid sulfate soil area of Vietnam. **International Journal of Systematic and Evolutionary Microbiology**, Reading, v. 60, n. Pt 4, p. 764-768, 2010. PMid:19656936. http://dx.doi.org/10.1099/ijs.0.011569-0.

LI, X.; QIAN, J.; WANG, C.; ZHENG, K.; YE, L.; FU, Y.; HAN, N.; BIAN, H.; PAN, J.; WANG, J.; ZHU, M. Regulating cytoplasmatic calcium homeostasis can reduce aluminum toxicity in yeast. **PLoS**

One, San Francisco, v. 6, n. 6, p. e21148, 2011. PMid:21698264. http://dx.doi.org/10.1371/journal.pone.0021148.

LIM, P. H.; PISAT, N. P.; GADHIA, N.; PANDEY, A.; DONOVAN, F. X.; STEIN, L.; SALT, D. E.; EIDE, D. J.; MACDIARMID, C. W. Regulation of Alr1 mg transporter activity by intracellular magnesium. **PLoS One**, San Francisco, v. 6, n. 6, p. e20896, 2011. PMid:21738593. http://dx.doi.org/10.1371/journal.pone.0020896.

MACDIARMID, C. W.; GARDNER, R. C. Al toxicity in yeast (a role for Mg?). **Plant Physiology**, Minneapolis, v. 112, n. 3, p. 1101-1109, 1996. http://dx.doi.org/10.1104/pp.112.3.1101.

MACDIARMID, C. W.; GARDNER, R. C. Overexpression of *Saccharomyces cerevisiae* magnesium transport system confers resistance to aluminum íon. **The Journal of Biological Chemistry**, Baltimore, v. 273, n. 3, p. 1727-1732, 1998. PMid:9430719. http://dx.doi.org/10.1074/jbc.273.3.1727.

MARIANO-DA-SILVA, S. **Acúmulo de cádmio por Saccharomyces cerevisiae fermentando mosto de caldo de cana**. 1998. 52 f. Dissertação (Mestrado em Microbiologia Agrícola)-Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 1998.

MARIANO-DA-SILVA, S.; BASSO, L. C. Efeitos do cádmio sobre o crescimento das Leveduras *Saccharomyces cerevisiae* PE-2 e *Saccharomyces cerevisiae* IZ-1904, e a capacidade da vinhaça em atenuar a toxicidade. **Ciência e Tecnologia de Alimentos**, Campinas, v. 24, n. 1, p. 16-22, 2004. http://dx.doi.org/10.1590/S0101-20612004000100004.

MARIANO-DA-SILVA, S.; BRAIT, J. D. A.; ANGELONI, L. H. P.; MARIANO-DA-SILVA, F. M. S.; LEITE, C. A. O.; BRAGA, P. Effects of nickel on the *Saccharomyces cerevisiae* Fleishmann mineral composition. **Ciência e Tecnologia de Alimentos**, Campinas, v. 27, n. 3, p. 490-494, 2007. http://dx.doi.org/10.1590/S0101-20612007000300013.

MARIANO-DA-SILVA, S.; OLIVEIRA, S. L.; LEITE, C. A. O.; PRADO, R. S.; FARIA, F. P.; OLIVEIRA, R. C. N.; MARIANO-DA-SILVA, F. M. S. Effect of pH, dextrose and yeast extract on cadmium toxicity on *Saccharomyces cerevisiae* PE-2. **Ciência e Tecnologia de Alimentos**, Campinas, v. 29, n. 2, p. 295-299, 2009. http://dx.doi.org/10.1590/S0101-20612009000200009.

OLIVEIRA, R. P. S.; BASSO, L. C.; PESSOA-JUNIOR, A.; PENNA, T. C. V.; BORGUI, M.; COVERTI, A. Response of *Saccharomyces cerevisiae* to cadmium and nickel stress. The use of the sugar cane vinasse as a potential mitigator. **Biological Trace Element Research**, London, v. 145, n. 1, p. 71-80, 2012. PMid:21809054. http://dx.doi.org/10.1007/s12011-011-9156-0.

OLIVEIRA, R. P. S.; TORRES, B. R.; ZILLI, M.; MARQUES, C. A. V.; BASSO, L. C.; COVERTI, A. Use of sugar cane vinasse to mitigate aluminum toxicity to *Saccharomyces cerevisiae*. **Archives of Environmental Contamination and Toxicology**, New York, v. 57, n. 3, p. 590-596, 2009. PMid:19198750.

PAGANI, A.; MALLARINO, A. P. Soil pH and crop grain yield as affected by the source and rate of lime. **Soil Science Society of America Journal**, Madison, v. 76, n. 5, p. 1877-1886, 2012. http://dx.doi.org/10.2136/sssaj2012.0119.

REHMUS, A.; BIGALKE, M.; VALAREZO, C.; CASTILHO, J. M.; WILCKE, W. Aluminum toxicity to tropical montane forest tree seedlings in southern Ecuador: response of biomass and plant morphology to elevated Al concentrations. **Plant and Soil**, The Hague, v. 382, n. 1, p. 301-315, 2014. http://dx.doi.org/10.1007/s11104-014-2110-0.

RODRÍGUES-PORRATA, B.; NOVO, M.; GUILLAMÕN, J.; ROZËS, N.; MAS, A.; CORDERO-OTERO, R. C. Vitality enhancement of the rehydrated active dry wine yeast. **International Journal of Food Microbiology**, Amsterdam, v. 126, n. 1-2, p. 116-122, 2008. PMid:18619697. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.05.016.

SCHOTT, E. J.; GARDNER, R. C. Aluminum-sensitive mutants of *Saccharomyces cerevisiae*. **Molecular & General Genetics**, Berlin, v. 254, n. 1, p. 63-72, 1997. PMid:9108291. http://dx.doi.org/10.1007/s004380050391.

SCHWALFENBERG, G.; GENIUS, S. J.; RODUSHKIN, I. The benefits and risks of consuming brewed tea: beware of toxic element contamination. **Journal of Toxicology**, Cairo, v. 2013, n. 1, p. 1-8, 2013. PMid:24260033. http://dx.doi.org/10.1155/2013/370460.

SHAW, C. A.; TOMLJENOVIC, L. Aluminum in the Central Nervous System (CNS): toxicity in humans and animal, vaccine adjuvants, and autoimmunity. **Immunologic Research**, Basel, v. 56, n. 2-3, p. 204-316, 2013. PMid:23609067. http://dx.doi.org/10.1007/s12026-013-8403-1.

SOUZA, R. B. **Análise do desempenho fermentativo da levedura Saccharomyces cerevisiae em resposta a composição mineral do meio**. 2012. 66 f. Dissertação (Mestrado em Biotecnologia)-Faculdade de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, 2012.

TREVELYAN, W. E.; HARRISON, J. S. Studies on yeast metabolism. 5. The trehalose content of baker's yeast during anaerobic fermentation. **The Biochemical Journal**, London, v. 62, n. 2, p. 177-183, 1956a. PMid:13293170. http://dx.doi.org/10.1042/bj0620177b.

TREVELYAN, W. E.; HARRISON, J. S. Studies on yeast metabolism. 7. Yeast carbohydrate fraction. Separation from nucleic acid analysis and behavior during anaerobic fermentation. **The Biochemical Journal**, London, v. 63, n. 1, p. 23-33, 1956b. PMid:13315242. http://dx.doi.org/10.1042/bj0630023.

TROFIMOVA, Y.; WALKER, G.; RAPOPORT, A. Anhydrobiosis in yeast: influence of calcium and magnesium ions on yeast resistance to dehydratation-rehydratation. **FEMS Microbiology Letters**, Amsterdam, v. 308, n. 1, p. 55-61, 2010. PMid:20487021.

WALKER, G. M. **Bioethanol**: science and technology of fuel alcohol. London: Bookboon.com Ltd., 2010. Available at: <http://

bookboon.com/en/bioethanol-science-and-technology-of-fuel-alcohol-ebook>. Accessed on: 03 aug. 2016.

WALKER, G. M.; SMITH, G.; HALL, N. The importance of metal ions in ethanolic fermentations. In: BRYCE, J.; STEWART, G. G. WORLDWIDE CONFERENCE OF DISTILLED SPIRITS, 1., 2003, Glasgow. **Proceedings...** Glasgow: Institute of Brewing, 2003. chap. 16, p. 113-119.

WOMACK, F. C.; COLOWICK, S. P. Proton – dependent inhibition of yeast and brain hexokinases by aluminum in ATP preparations. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 76, n. 10, p. 5080-5084, 1979. http://dx.doi.org/10.1073/pnas.76.10.5080.

YOSHINO, M.; YAMADA, Y.; MURAKAMI, K. Inhibition by aluminum íon of NAD – dependent and NADP – dependent isocitrate dehydrogenases from yeast. **The International Journal of Biochemistry**, Oxford, v. 24, n. 10, p. 1615-1618, 1992. PMid:1397488. http://dx.doi.org/10.1016/0020-711X(92)90178-4.