EFFECTS ON THE MINERAL COMPOSITION OF FLEISCHMANN'S YEAST (*Saccharomyces cerevisiae*) FERMENTING SUGAR-CANE JUICE CONTAMINATED WITH COBALT

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ABSTRACT

The current work aimed to study the accumulation and the effects of cobalt on the mineral composition of Fleischmann's yeast *Saccharomyces cerevisiae* fermenting sugarcane juice with controlled contamination, in sub-toxic levels of cobalt. The sterilized juice (120°C, 20 minutes), with 12% of total reducing sugars (TRS) and four levels of pH (3.5; 4.5; 5.5 and 6.5) was added with cobalt chloride salt ($CoCl_2.6H_2O$) resulting in six levels of contamination (0.0; 0.1; 0.5; 1.0; 2.0 e 5.0 mg Co L^{-1}) and 24 treatments. The juice inoculation was carried out with bread yeast (10% p/p). After the end of the fermentation (6 hours), the fermented media were centrifuged, and the cellular viability, budding rate and levels of cobalt, phosphorus, calcium, magnesium, sulfur, copper, iron, manganese, potassium and zinc were determined (in dry weight – dw). The absorption of cobalt was influenced by the pH. This absorption was higher in pH 3.5, whereas in pH 6.5 we found almost no absorption. The levels of potassium, calcium, zinc and phosphorus decreased with the increase of pH while the levels of sulfur content increased. No significant differences could be observed in the fermentative velocity for the several treatments.
KEYWORDS: bioaccumulation, heavy metal, pH.

EFEITOS NA COMPOSIÇÃO MINERAL DA LEVEDURA FLEISCHMANN (*Saccharomyces cerevisiae*) FERMENTANDO CALDO DE CANA CONTAMINADO COM COBALTO

RESUMO

O presente trabalho teve por finalidade estudar o acúmulo e os efeitos do cobalto na composição mineral da levedura Fleichmann *Saccharomyces cerevisiae* fermentando mosto de caldo de cana com contaminações controladas, em níveis sub-tóxicos, do citado metal. O mosto esterilizado (120ºC, 20 minutos), com 12% de açúcares re-

dutores totais (ART) e quatro níveis de pH (3,5; 4,5; 5,5 e 6,5), foi acrescido com o sal cloreto de cobalto ($CoCl_2.6H_2O$) resultando em seis níveis de contaminações (0,0; 0,1; 0,5; 1,0; 2,0 e 5,0 mg $Co.L^{-1}$) e 24 tratamentos. A inoculação do mosto foi executada com fermento de panificação (10% p/p). Após o término da fermentação (6 horas) os meios de fermentação foram centrifugados, sendo determinadas a viabilidade celular, a taxa de brotamento e teores de cobalto, fósforo, potássio, cálcio, magnésio, enxofre, cobre, ferro, manganês e zinco (em matéria seca). A absorção do cobalto foi influenciada pelo pH, sendo maior em pH 3,5 enquanto em pH 6,5 praticamente não houve absorção. Os níveis de potássio, fósforo, cálcio e zinco decresceram com o aumento do pH, sendo que os níveis de enxofre aumentaram. Não foi observada diferença significativa na velocidade fermentativa entre os diferentes tratamentos.
PALAVRAS-CHAVE: bioacumulação, metal pesado, pH.

INTRODUCTION

When sugarcane, the raw material for ethanol fuel production, is derived from acid soils, place where the absorption and accumulation of heavy metals by plants is facilitated (CHIBUIKE; OBIORA, 2014), considerable quantities of these metals can be encountered in the plant stem (CHIBUIKE; OBIORA, 2014; PANDEY et al., 2016), and transported to the fermentative process (XIE et al., 2014).

The Melle-Boinot method is the traditional biotechnological process of ethanol fuel production employed in Brazil, in which yeast cells are recovered by centrifugation and re-used, increasing ethanol yields and leading the yeast to a peculiar physiological condition that might provide metal accumulation (MARIANO-DA-SILVA et al., 2016). Yeast cells growing in a medium of fermentation containing concentrations of metals, even in sub-toxic levels, may accumulate and present higher concentration of these metals when compared to those found in the juice, due to the phenomenon of bioaccumulation (ALI IMAM et al., 2016).

The absorption of cobalt (Co) by yeast cells depends on 1) the amount of glucose, 2) the initial pH level 3) the concentration of Co in the juice and 4) the metabolism of the yeast cells, considering that, dead cells are not capable of accumulating Co, releasing it to the media when killed (HELDWEIN et al., 1977; GALEDAR; YOUNESI, 2013; SAAD, 2015). After the absorption, the Co^{+2} ions are transported through the membrane cell, probably by membrane transport proteins, until the cytosol, where they are bonded to metalloproteins in the vacuole (HELDWEIN et al, 1977).

Even though some heavy metals may act as essential cofactors and have important roles in yeast cells, their concentration, if high, may cause problems. These metals may damage the yeast-cell membrane, alter enzyme and protein function, cause oxidative stress, DNA damage and lipid peroxidation (ASSMANN et al., 1996; HOSINER et al., 2014). They may also damage the H^+ ATPase enzyme, a key component in the plasmatic membrane of yeast cells. This enzyme creates an electrochemical gradient of protons through the membrane that controls the transportation of several organic and inorganic solutes, such as sugar. The H^+ ATPase enzyme is clearly responsible for several physical, chemical and nutritional facts, and it is usually an indicator of stress, including the one caused by heavy metals (ASSMANN et al., 1996; CHAROENBHAKDI et al., 2016).

In this context, the goal of this work was to give a deeper knowledge about the influence of heavy metals, in this case Co, on the mineral composition of *Saccharomyces cerevisiae*. Besides helping to explain the mechanisms of toxicity of heavy metals, this knowledge is quite important for the manufacturing of biological filters for the detoxification of wastewater, the production of microbial biomass for animal nutrition and for the biotechnological process of ethanol fuel production.

MATERIAL AND METHODS

Material preparation

All reusable glassware items (glass, quartz, polyethylene, teflon, etc.) were prepared for use by being washed with detergent, rinsed with ultra pure water and soaked for four hours in a mixture of nitric acid, hydrochloric acid and water (1:2:9) followed by another rinse with ultra pure water and heat drying (MARIANO-DA-SILVA et al., 2016);

Yeast strain

Blocks of pressed yeast were stored at 3ºC ±1 for later use in the fermentation experiment

Sugar-cane must preparation

For the fermentation, sugar-cane juice was sterilized (autoclaved at 1ATM, 120 ºC for 20 minutes) and diluted with distilled (DI) water to 12 % of total reducing sugars (TRS). The juice had its pH adjusted to 3.5, 4.5, 5.5 and 6.5 with a solution of NaOH 0.1 M or H_2SO_4 0.1M according to the treatment. The juice was then added with cobalt chloride salt ($CoCl_2.6H_2O$) resulting in six contamination levels for each pH (0.0, 0.1, 0.5, 1.0, 2.0 and 5.0 mmol Co L^{-1}) totalizing an amount of 24 treatments

Fermentation experiment

Fermentations were carried out with sterilized (autoclaving at 1 ATM, 120^{o}C for 20 minutes) 250 mL of sugar-cane juice (diluted with distilled water to 16º Brix) and 25 g of bread yeast (wet weight – ww) in 500 mL Erlenmeyer flasks capped with aluminum foil and kept in thermostatic stove at 30ºC ±1. The end of fermentation, whose velocity was estimated by weight loss (CO_2) (each hour), occurred in 6 hours. As soon as the fermentation ended, the ferment was separated through centrifugation and submitted to analysis

Viable count, budding rate and bacterial contamination

After the fermentation, 0.5 ml of each yeast sample was tested. The samples were diluted, erythrosine stained and evaluated for yeast viability, budding rate and bacterial contamination (rod type cells) with microscope analysis (MARIANO-DA-SILVA et al., 2016)

Iron (Fe), cobalt (Co), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn) and zinc (Zn) quantification

Approximately 0.5 g of yeast sample was mineralized with 2:5:3 sulfuric acid (H_2SO_4), nitric acid (HNO_3) and perchloric acid ($HClO_4$) for digestion at 220 ºC for 3 hours. The mineral contents were measured using an atomic absorption

spectrophotometer, with acetylene flame atomization, in Perkin Helmer AAnalyst Model 100 Atomic Absorption Spectrophotometer (PETROZZI, 2013)

Sulfur (S) quantification

Approximately 0.5 g of yeast sample was mineralized with 2:1 hydrogen peroxide (H_2O_2) and perchloric acid ($HClO_4$) for digestion at 220 ºC for 3 hours. The mineralized sample was dissolved to a concentration of 1:100 in distilled (DI) water. Later, 1 mL of 6 M chloridric acid (HCl) and 0.5g of barium chloride ($BaCl_2$) were added. After 5 minutes, the color was measured in the spectrophotometer at 420 nm (PETROZZI, 2013)

Phosphorus (P) quantification

Approximately 0.5 g of yeast sample was mineralized with 2:1 hydrogen peroxide (H_2O_2) and perchloric acid ($HClO_4$) for digestion at 220 ºC for 3 hours. The mineralized sample was dissolved to a concentration of 1:100 in DI water. Later, 2mL of 0.25% ammonium metavanadate (NH_4VO_3) and 2mL of 5% ammonium molybdate ($[NH_4]_2MoO_4$) were added. After 15 minutes, the color was measured using the Analyzer colorimeter at 660 nm (PETROZZI, 2013)

Statistical analysis

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

RESULTS AND DISCUSSION

The absorption of Co was influenced by pH, being this absorption higher in pH 3.5, while there was almost no absorption in pH 6.5 (Table 1). In higher levels of pH, Co oxides and hydroxides are formed with decreasing quantities of free and available ions for the interaction with the yeast decreasing then the absorption and toxic signs (GALEDAR; YOUNESI, 2013). Roughly speaking, the intracellular rates of Co increased with metal increment in the growth medium. This tendency was also observed by Galedar e Younesi (2013) in *Saccharomyces cerevisiae* strains susceptible to that metal toxicity.

TABLE 1 – Cobalt content in yeast (mmol kg^{-1} in dw)

Treatment	Cobalt (mmol Co L^{-1})					
pH level	0.00	0.10	0.50	1.00	2.00	5.00
3.5	0.0226^{Ea}	1.9598^{Ea}	9.0498^{Da}	21.4310^{Ca}	33.6539^{Ba}	45.2490^{Aa}
4.5	0.0023^{Ca}	1.9344^{Ca}	6.6120^{Ba}	7.5679^{Bb}	10.1131^{Bb}	18.6369^{Ab}
5.5	0.0147^{Ca}	0.7523^{BCa}	2.0645^{BCb}	3.3767^{BCbc}	4.7115^{Bc}	12.5849^{Ac}
6.5	0.0082^{Ba}	4.9604^{ABa}	1.8382^{ABb}	0.7919^{Abc}	0.5656^{Abc}	0.4355^{Ad}
STANDARD DEVIATION: 20.008 (%)						

Averages followed by same letters (capital on the same column or small on the same line) are not significantly different according Tukey's HSD multi comparison tests (Alpha = 1%)

The intracellular content of K tended to decrease with the increase of Co contents in the growth medium in all tested pH levels (Table 2). It is also likely that pH influences this tendency, considering that the decrease in pH levels goes along with the decrease in the intracellular content of K. These results are in accordance with those reported by several authors (BRADY; DUNCAN, 1994; ASSMANN et al., 1996; GALEDAR; YOUNESI, 2013).

TABLE 2 – Potassium content in yeast (mmol kg^{-1} in dw)

Treatment	Potassium (mmol Co L^{-1})					
pH level	0.00	0.10	0.50	1.00	2.00	5.00
3.5	0.3427^{Bb}	0.3223^{Bb}	0.3282^{Bc}	0.3257^{Bb}	0.3236^{Bb}	0.2805^{Cc}
4.5	0.3495^{Ab}	0.3359^{Aab}	0.3308^{Abc}	0.3333^{Ab}	0.3257^{Ab}	0.3248^{Ab}
5.5	0.4160^{Aa}	0.4033^{Aba}	0.3623^{Bab}	0.3393^{Cb}	0.3221^{Cb}	0.3427^{Cab}
6.5	0.4808^{Aa}	0.4220^{Ba}	0.4203^{Ba}	0.4092^{Cba}	0.3956^{Ca}	0.3998^{Ca}
STANDARD DEVIATION: 7.137 (%)						

Averages followed by same letters (capital on the same column or small on the same line) are not significantly different according Tukey's HSD multi comparison tests (Alpha = 1%)

Heavy metals may cause the fluidization of the yeast-cell membrane, inducing K channels to open and intracellular K levels to decrease (ASSMANN et al., 1996; MARIANO-DA-SILVA et al., 2007). They also act directly on the SH- groups of K exit-channels, causing them to open with consequent K loss (ASSMANN et al., 1996). Moreover, heavy metals accumulation in the vacuole may cause Ca displacement from the vacuole bindings leading to an increasing of free Ca^{+2} ions in the cytosol, which may induce K exit channels to open, allowing its loss (K) to the surrounding media (ASSMANN et al., 1996; BLACKWEEL; TOBIN, 1999).

Although Mariano-da-Silva et al. (2007) have reported that Ni does not affect the intracellular levels of phosphorus, phosphorus levels diminished with the increase of Co contents in the media (Table 3). It is likely that pH also influences this tendency since the decrease in pH levels goes along with the decrease in the content of intracellular P.

TABLE 3 – Phosphorus content in yeast (mmol kg^{-1} in dw)

Treatment	Phosphorus (mmol Co L^{-1})					
pH level	0.00	0.10	0.50	1.00	2.00	5.00
3.5	0.2238^{Aa}	0.2023^{ABb}	0.2077^{ABb}	0.2077^{ABb}	0.1880^{Bb}	0.1647^{Cb}
4.5	0.2475^{Aa}	0.2454^{Aab}	0.2195^{Bb}	0.2173^{Ba}	0.2077^{Ba}	0.2069^{Bba}
5.5	0.2637^{Aa}	0.2540^{Aa}	0.2441^{Aab}	0.2680^{Aa}	0.2690^{Aa}	0.2744^{Aa}
6.5	0.2615^{Aa}	0.2637^{Aa}	0.2647^{Aa}	0.2540^{Aa}	0.2529^{Aa}	0.2594^{Aa}
STANDARD DEVIATION: 7,048 (%)						

Averages followed by same letters (capital on the same column or small on the same line) are not significantly different according Tukey's HSD multi comparison tests (Alpha = 1%)

The intracellular levels of Ca seem to decrease with the increase of Co levels in the growth medium (Table 4). As reported by Assmann et al. (1996) and Blackweel e Tobin, 1999 for Cd, and Mariano-da-Silva (2007) for Ni, Co affected the intracellular contents of Ca. Probably the heavy metals accumulation in the vacuole causes the displacement of Ca from their vacuolar storage sites, increasing Ca^{+2} concentrations in the cytosol. As the plasmatic membrane is damaged by Co, fluidization is triggered, and the transport channels are opened, causing a reduction in the levels of intracellular free-ions (BLACKWEEL; TOBIN, 1999).

TABLE 4 – Calcium content in yeast (mmol kg^{-1} in dw)

Treatment	Calcium (mmol Co L^{-1})					
pH level	0.00	0.10	0.50	1.00	2.00	5.00
3.5	13.7226^{Ab}	14.1384^{Ab}	12.8077^{Ab}	12.3819^{Ac}	12.2572^{Ac}	12.8909^{Ac}
4.5	12.0592^{Ab}	12.0592^{Ab}	14.1384^{Ab}	13.7226^{Ac}	14.5346^{Ac}	11.4355^{Ac}
5.5	21.8313^{Aa}	22.8709^{Aa}	20.7917^{Aa}	20.7917^{Aab}	20.3759^{Aa}	15.8017^{Bab}
6.5	25.7818^{Aa}	22.2472^{Aa}	24.3263^{Aa}	23.0788^{Aa}	21.4155^{Aa}	20.5838^{Aa}
STANDARD DEVIATION: 11.161 (%)						

Averages followed by same letters (capital on the same column or small on the same line) are not significantly different according Tukey's HSD multi comparison tests (Alpha = 1%)

It is also possible to notice that the intracellular contents of Ca are affected by the pH in the growth medium, having a tendency to increase in the higher pH levels. According to Brady e Duncan (1994), this behavior is due to the high extracellular concentrations of H^+ found in low pH levels.

Although Mariano-da-Silva et al. (2007) have reported that Ni did not affect the intracellular contents of S, the S contents were higher in the treatments with pH 3,5 and tended to increase as Co content increased in the growth medium (Table 5).

TABLE 5 – Sulfur content in yeast (mmol kg^{-1} in dw)

Treatment	Sulfur (mmol Co L^{-1})					
pH level	0.00	0.10	0.50	1.00	2.00	5.00
3.5	16.0152^{Aa}	16.5596^{Ba}	16.7033^{Ba}	18.058^{BCa}	19.556^{ABa}	19.2348^{Aa}
4.5	16.3236^{Aa}	14.0362^{Aa}	13.9322^{Aa}	20.2745^{Aa}	20.7943^{Aa}	19.1790^{Aa}
5.5	12.9955^{Ab}	15.0759^{Aa}	15.0759^{Aa}	17.1553^{Aa}	17.1553^{Aa}	18.5189^{Aa}
6.5	10.9170^{Ab}	8.3177^{Aab}	10.3972^{Ab}	9.3575^{Ab}	8.3177^{Ab}	14.0362^{Aab}
STANDARD DEVIATION: 21.859 %						

Averages followed by same letters (capital on the same column or small on the same line) are not significantly different according Tukey's HSD multi comparison tests (Alpha = 1%)

The levels of Mn (Table 6) decreased with the increase of pH, showing almost no difference among Co concentrations (Table 1). Mariano-da-Silva et al. (2007), investigating the effects of Ni on the mineral composition of *Saccharomyces cerevisiae*, reported that, in all the tested concentrations, this metal affected the cellular concentrations of manganese.

TABLE 6 – Manganese content in yeast (mmol kg^{-1} in dw)

Treatment	Manganese (mmol Co L^{-1})					
pH level	0.00	0.10	0.50	1.00	2.00	5.00
3.5	0.5760^{Aa}	0.5897^{Aa}	0.5349^{Aa}	0.5623^{Aa}	0.4937^{Aa}	0.4114^{Aa}
4.5	0.5623^{Aa}	0.5349^{Aa}	0.4526^{Aab}	0.4937^{Aab}	0.4800^{Aa}	0.4389^{Aa}
5.5	0.2743^{Ab}	0.2606^{Ab}	0.2606^{Ac}	0.2606^{Ac}	0.2194^{Ab}	0.2057^{Ab}
6.5	0.3566^{Ab}	0.3292^{Ab}	0.3703^{Abc}	0.3840^{Ab}	0.3977^{Aa}	0.4389^{Aa}
STANDARD DEVIATION: 11.102 (%)						

Averages followed by same letters (capital on the same column or small on the same line) are not significantly different according Tukey's HSD multi comparison tests (Alpha = 1%)

The intracellular contents of Zn seem to decrease with the decrease of pH and to increase with higher Co concentrations present in the growth media (Table 7). Ting e Teo (1995), who studied the absorption of Cd and Zn by yeasts, have reported a different phenomenon. The authors state that Cd does not affect Zn absorption instantly, however inhibiting it in the long term.

TABLE 7 – Zinc content in yeast (mmol kg^{-1} in dw)

Treatment	Zinc (mmol Co L^{-1})					
pH level	0.00	0.10	0.50	1.00	2.00	5.00
3.5	1.1725^{Ac}	1.1776^{Ab}	1.1266^{Ac}	1.2948^{Ac}	1.3050^{Ac}	0.6915^{Bc}
4.5	2.6151^{Ab}	2.5998^{Aa}	2.3449^{Ab}	2.3041^{Ab}	2.3143^{Aab}	2.3245^{Ab}
5.5	3.2217^{Aa}	3.0637^{Aa}	3.0790^{Aa}	3.0637^{Aab}	2.9617^{Aa}	2.8292^{Aab}
6.5	3.4205^{Aa}	3.2625^{Aa}	3.3135^{Aa}	3.4460^{Aa}	3.5014^{Abc}	3.5836^{Aa}
STANDARD DEVIATION: 14.220 (%)						

Averages followed by same letters (capital on the same column or small on the same line) are not significantly different according Tukey's HSD multi comparison tests (Alpha = 1%)

Despite the fact that Blackweel e Tobin (1999) and Mariano-da-Silva et al. (2007) have reported a decrease in the intracellular contents of Mg in yeasts submitted to fermentation in media with Cd and Ni, respectively, Co did not affect the intracellular contents of Mg in the tested concentrations (Table 8).

TABLE 8 – Magnesium content in yeast (mmol kg^{-1} in dw)

Treatment	Magnesium (mmol Co L^{-1})					
pH level	0,00	0,10	0,50	1,00	2,00	5,00
3.5	53,0755	51,7040	45,3953	53,1441	56,2299	51,4297
4.5	79,5447	76,1160	76,4589	73,0302	71,6588	69,2587
5.5	60,3442	57,9442	56,5727	57,6013	55,5441	52,1155
6.5	50,4012	51,4297	53,4869	53,1441	54,5155	51,0869
STANDARD DEVIATION: 6,732 (%)						

Averages were not significantly different according to ANOVA at 1% confidence level

Although Alkim et al. (2013) have reported that resistance to cobalt is related to some mechanisms of intracellular reallocation of Fe and the use of Co, by some cellular proteins, instead of Fe for their catalytic activities, it seems not to have any influence on the cellular contents of this metal in the tested concentrations (Table 9).

TABLE 9 – Iron content in yeast (mmol kg^{-1} in dw)

Treatment	Iron (mmol Co L^{-1})					
pH level	0,00	0,10	0,50	1,00	2,00	5,00
3.5	10,6853	9,7203	8,8781	10,1941	9,5273	12,2644
4.5	13,2996	17,5983	11,5626	12,0714	11,9662	13,5979
5.5	12,1767	9,4571	8,7904	9,7028	8,4921	9,3870
6.5	13,2821	12,4048	12,6154	13,1768	9,9835	14,9489
STANDARD DEVIATION: = 21,819 (%)						

Averages were not significantly different according to ANOVA at 1% confidence level

Similarly to the contents of Fe, the intracellular contents of Cu were not affected by Co in the tested concentrations. According to Alesso et al. (2015), the pathway components and the proteins involved in the Fe and Cu absorption by *Saccharomyces cerevisiae* were similar. Okuyama et al. (1999) had already reported that the inhibitory concentrations of Co had almost not affected the accumulation of copper in yeast cells.

TABLE 10 – Copper content in yeast (mmol kg^{-1} in dw)

Treatment	Copper (mmol Co L^{-1})					
pH level	0,00	0,10	0,50	1,00	2,00	5,00
3.5	0,1626	0,1416	0,1731	0,1416	0,1364	0,1416
4.5	0,1626	0,1521	0,1469	0,1574	0,1469	0,1364
5.5	0,1521	0,1521	0,1626	0,1574	0,1836	0,1679
6.5	0,1679	0,1679	0,1626	0,1731	0,1521	0,1783
STANDARD DEVIATION: 12,526 (%)						

Averages were not significantly different according to ANOVA at 1% confidence level

In tables 11 and 12, the results obtained for viability and budding rate can be observed. The viability rate decreases with pH decrease and cobalt content increase. The budding rate did not show expressive variations within the different treatments.

TABLE 11 - Viability rate (%)

Treatment	(mmol Co L^{-1})					
pH level	0,00	0,10	0,50	1,00	2,00	5,00
3.5	$88,34^{Ab}$	$82,98^{Ac}$	$76,12^{Bd}$	$72,60^{Cd}$	$70,21^{Cd}$	$66,90^{Dd}$
4.5	$99,12^{Aa}$	$95,56^{Bb}$	$89,00^{Cc}$	$84,29^{Cc}$	$80,78^{Dc}$	$76,90^{Ec}$
5.5	$99,77^{Aa}$	$98,92^{Aa}$	$95,56^{ABb}$	$90,67^{Bb}$	$89,39^{Bb}$	$87,89^{Bb}$
6.5	$99,56^{Aa}$	$99,20^{Aa}$	$99,67^{Aa}$	$98,90^{Aa}$	$99,54^{Aa}$	$98,07^{Aa}$
STANDARD DEVIATION: 2,37 (%)						

Averages followed by same letters (capital on the same column or small on the same line) are not significantly different according Tukey's HSD multi comparison tests (Alpha = 1%).

TABLE 12 - Budding rate (%)

Treatment	(mmol Co L^{-1})					
pH level	0,00	0,10	0,50	1,00	2,00	5,00
3.5	20,89	19,20	19,43	21,12	19,84	20,98
4.5	21,90	18,16	20,98	21,34	21,32	20,94
5.5	20,93	20,67	21,00	19,56	18,76	21,50
6.5	21,90	19,65	19,67	18,99	20,01	19,87
STANDARD DEVIATION: 12,90 (%)						

Averages were not significantly different according to ANOVA at 1% confidence level.

The viability rates were strongly correlated with cobalt accumulation and cobalt availability. Mariano-da-Silva et al., (2007) obtained similar results working with nickel, demonstrating that the metal accumulation is an attempt of detoxification. It was not possible to verify significant differences in the fermentative velocity in the different cobalt rates for the different levels of pH (Figures 1A, B, C e D). Nevertheless, in pH 3.5 (Figure 1), there were significant differences in the distinct treatments, making it possible to notice that the velocity increased with increments in the cobalt rates.

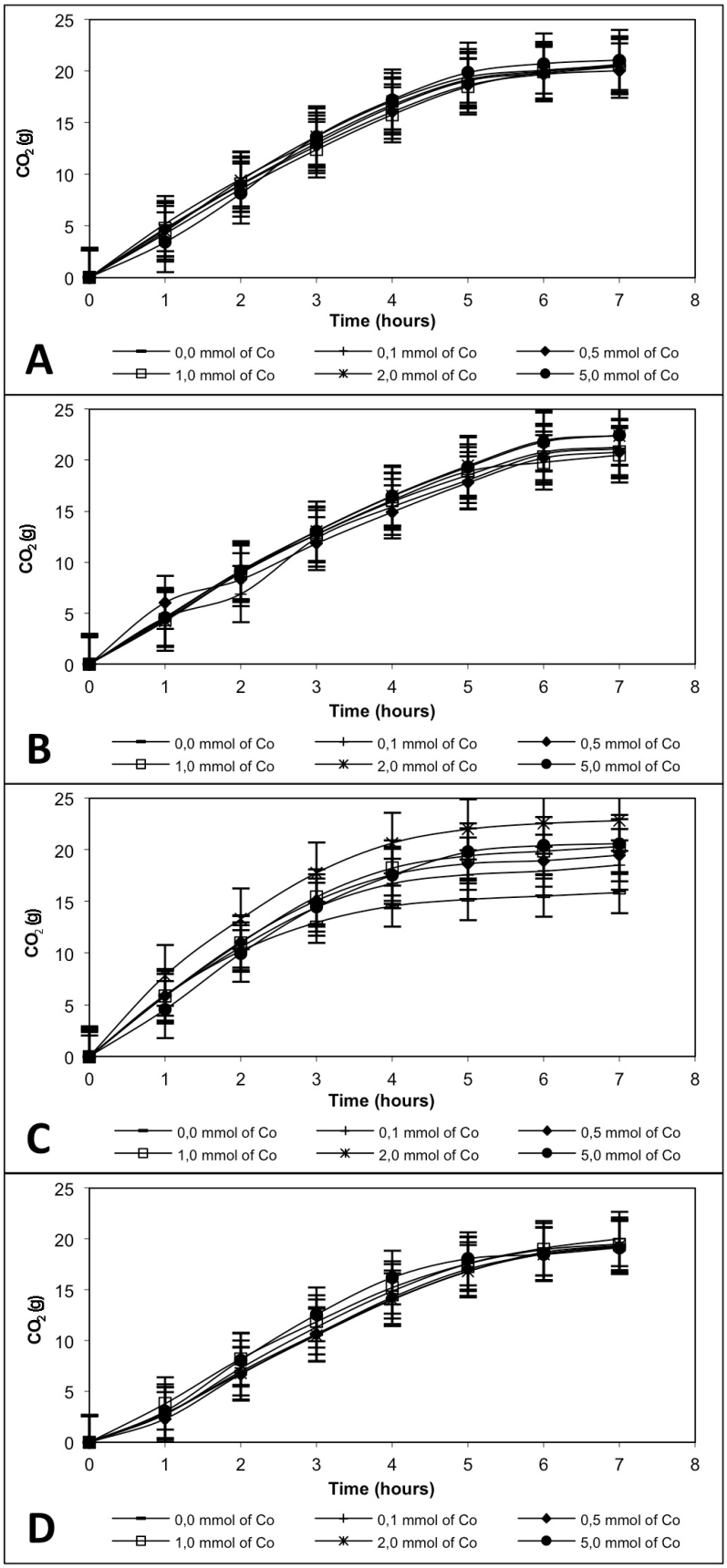

FIGURE 1 – CO_2 produced in pH 3.5 (A), pH 4.5 (B), pH 5.5 (C) e pH 6.5 (D).

CONCLUSIONS

The pH influenced Co toxicity on *Saccharomyces cerevisiae* altering its viability and mineral composition. At the highest pH level (6.5), Co was almost nontoxic to the yeast since it was in an unavailable form to be taken up by the yeast. With decrease in pH, an accumulation of Co was encountered, leading to a decrease in the cellular viability and in the cellular levels of K, P, Ca and Zi.

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