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Effects of nickel on the mineral composition of Fleischmann's yeast (*Saccharomyces cerevisiae*)

Efeitos do níquel na composição mineral da levedura Fleischmann (Saccharomyces cerevisiae)

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Abstract

Sugar cane juice containing 12% (w.w $^{-1}$) of total reducing sugars and 0.0 to 5.0 mmol of nickel L^{-1}, with pH ranging from 3.5 to 6.5, was inoculated with Fleischmann's yeast (*Saccharomyces cerevisiae*) (10% w.w $^{-1}$). Six hours after fermentation, the yeast's cellular viability and trehalose content were evaluated. The resulting must was centrifuged and the raw yeast was analyzed by atomic absorption spectroscopy to evaluate the intracellular levels of calcium, copper, iron, magnesium, manganese, nickel, phosphorus, potassium, sulfur and zinc. The intracellular levels of iron, magnesium and calcium were affected and the yeast's susceptibility to nickel was enhanced by the decrease in pH. The yeast's growth was not affected by nickel at high pH, but the toxic effects of nickel were potentiated at low pH.

Keywords: nickel; mineral composition; *Saccharomyces cerevisiae*; yeast.

Resumo

O presente trabalho teve por finalidade estudar o acúmulo e os efeitos do níquel na composição mineral da levedura *Saccharomyces cerevisiae* Fleichmann fermentando mosto de caldo de cana com contaminações controladas, em níveis subtóxicos, do citado metal. O mosto esterelizado (120 °C/20 minutos), com 12% de açúcares redutores totais (ART) e quatro níveis de pH (3,5; 4,5; 5,5 e 6,5), foi acrescido com o sal cloreto de níquel ($NiCl_2.6H_2O$), resultando em seis níveis de contaminações (0,0; 0,1; 0,5; 1,0; 2,0 e 5,0 mmol $Ni.L^{-1}$) e 24 tratamentos. A inoculação do mosto foi executada com fermento de panificação (10% $p.p^{-1}$). Depois de 6 horas de fermentação, as leveduras foram obtidas por centrifugação, para posterior análise da viabilidade celular, dos teores de trealose, bem como dos teores celulares de níquel, fósforo, potássio, cálcio, magnésio, enxofre, cobre, ferro, manganês e zinco. O níquel parece influenciar nos teores intracelulares de ferro, magnésio e cálcio, sendo que a toxidade do níquel foi potencializa com o decréscimo do pH.

Palavras-chave: composição mineral; levedura; níquel; *Saccharomyces cerevisiae*.

1 Introduction

Several studies have focused on the toxicity of heavy metals, particularly the toxicity associated with the phenomenon of bioaccumulation[34]. Several heavy metals are essential to the metabolism of microorganisms, since they are required in numerous enzymatic reactions and protein compositions. On the other hand, metal elements can be highly toxic and may disturb the microorganic metabolism.

Heavy metals comprise about 40 elements, including nickel, which has a minimum density of 6.0 $g.cm^{-3}$ [2]. Nickel is the 28th element in the periodic table, with an atomic weight of 58.71 $g.cm^{-3}$ and specific density of 8.91[1]. This metal, which is usually present in soils next to mineral lodes or in deposits of industrial residues (e.g., galvanoplastic residues containing nickel), is easily absorbed by plants[10,21,23,37]. Nickel can even percolate into agricultural soils through server slime, through discarded cement waste, accumulated wastes from burned fossil fuel and urban garbage, among others[1,2,36]. The average world values of nickel found in agricultural soils range from 20 to 40 ppm. However, MALAVOLTA[22] found 127 ppm in a structured Terra Roxa (purple soil) in the state of São Paulo.

The fermentation process normally employed in Brazil is the traditional Melle-Boinot method. Batch fermentation with total cell recycling keeps the cellular concentration high, increasing the alcohol content due to the low consumption of sugar for cell growth[19,20]. Due to the phenomenon of bioaccumulation, yeasts that grow by fermentation in the presence of heavy metals, even in subtoxic concentrations, may present higher cellular concentrations of these metals than those found in must[24,26-28,32]. This problem is believed to be aggravated when associated with yeast recycling[25].

Nickel absorption by yeast depends on the sugar and metabolism, considering that dead cells do not absorb metal[14]. After absorption, the cations are transported through the cell membrane, probably by cation transporting proteins, to the cytosol, where they are linked to metalloproteins in the vacuole[6,32,35,45].

Heavy metals may damage the yeast cell membrane by fluidizing it, which causes potassium channels to open and its intracellular level to decrease[4,9,16].

Accumulation of heavy metals in the vacuole may cause calcium displacement from the vacuole, increasing free Ca^{+2} ions in the cytosol, which may cause the potassium exit channels to open, with loss of potassium to the surrounding medium[4]. Mg^{+2} ions are also displaced from the vacuole, passing to the cytoplasm and to the extracellular medium[5]. Heavy metals

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may also act directly on the SH- groups of the potassium exit channels, causing them to open and resulting in loss of potassium[4]. H^+-ATPase damage is also reportedly caused by heavy metals in yeast[15].

A key component in the plasmatic membrane is H^+-ATPase, which creates an electrochemical gradient through the membrane that controls the transportation of a variety of inorganic and organic solutes, such as sugar[8,11].

This enzyme is clearly responsible for many physical, chemical and nutritional facts, and is usually a stress indicator, including the stress caused by heavy metals[4]. Heavy metals may also affect the sugar entering the yeast cell when they are linked to the anionic bindings of the plasmatic membrane. Several authors[31,42,43] have reported that sugar transport into the yeast cell is inhibited by heavy metals and this inhibition may be involved in these metals' (e.g., nickel, cobalt and cadmium) interaction with the polyphosphate membrane, causing a conformational change in some active sites, which would render binding with the sugar unviable.

2 Materials and methods

2.1 Material preparation

All reusable items (glass, quartz, polyethylene, Teflon, etc.) were prepared for use by washing with detergent in ultra pure water and soaking for 4 hours in a mixture of nitric acid, hydrochloric acid and water (1 + 2 + 9), followed by rinsing with ultra pure water and heat drying[29].

2.2 Yeast strain

Blocks of pressed yeast were stored at 3 °C ⇔1 for later use in the fermentation experiment.

2.3 Preparation of sugar-cane juice

For the fermentation, the juice was sterilized (1 ATM/20 minutes/121 °C) and diluted with sterilized distilled water to 12% of TRS (total reducing sugars). The pH was adjusted to 3.5; 4.5; 5.5 and 6.5, according to the treatment, through the addition of NaOH 0.1 M or H_2SO_4 0.1 M. Nickel chloride salt ($NiCl_2.6H_2O$) was then added to the musts, resulting in six levels of contamination (0.0, 0.1, 0.5, 1.0, 2.0 and 5.0 mmol Ni L^{-1}) and 24 treatments.

2.4 Fermentation experiment

Fermentation batches were prepared with 250 mL of sterilized (autoclaved at 1 ATM, 120 °C, 20 minutes) sugar cane must growth medium (diluted with distilled water to 16 °Brix) and 25 g of yeast (uw) in 500 mL Erlenmeyer flasks, which were capped with aluminum foil and placed in a thermostatic oven at 30 °C ⇔1. Fermentation, which was estimated by weight loss (CO_2) measured at 1 hour intervals, was concluded in 6 hours, at which point the fermented product was separated by centrifugation and analyzed.

2.5 Viable count, budding rate and bacterial contamination

After 18 hours of 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) by microscopic analysis, as described by AMORIM et al[3].

2.6 Yeast trehalose

Trehalose was extracted from 60 mg of washed cells (fresh wt) with 2 mL of 0.5 mol L^{-1} trichloroacetic acid in ice bath for 20 minutes (the suspension was shaken frequently), centrifuged[40,41], and 0.2 mL of each supernatant was subjected to anthrone reaction, according to Brin[7].

2.7 Nickel, potassium, calcium, magnesium, copper, manganese, and zinc quantification

Approximately 0.5 g of yeast sample was mineralized by 2:5:3 sulfuric acid (H_2SO_4), nitric acid (HNO_3) and perchloric acid ($HClO_4$) digestion at 220 °C for 3 hours. The mineral content was measured by atomic absorption spectrophotometry, with atomization by acetylene flame, in a Perkin Elmer model Analyst 100 atomic absorption spectrophotometer[44].

2.8 Sulfur quantification

Approximately 0.5 g of yeast sample was mineralized by 2:1 hydrogen peroxide (H_2O_2) and perchloric acid ($HClO_4$) digestion at 220 °C for 3 hours. The mineralized sample was dissolved 1:100 in distilled water, after which 1 mL of 6 M chloric acid (HCl) and 0.5 g of barium chloride ($BaCl_2$) were added. After 5 minutes, the color was measured in the spectrophotometer at 420 nm[30].

2.9 Phosphorus quantification

Approximately 0.5 g of yeast sample was mineralized by 2:1 hydrogen peroxide (H_2O_2) and perchloric acid ($HClO_4$) digestion at 220 °C for 3 hours. The mineralized sample was dissolved 1:100 in distilled water, and 2 mL of 0.25% ammonium metavanadate (NH_4VO_3) and 2 mL of 5% ammonium molybdate ($[NH_4]_2MoO_4$) were added. After 15 minutes, the color was measured with a colorimeter at 660 nm[30].

2.10 Nitrogen quantification

Approximately 0.5 g of yeast sample was mineralized by 2:1 hydrogen peroxide (H_2O_2) and perchloric acid ($HClO_4$) digestion at 220 °C for 3 hours. The nitrogen content was then determined by the Microkjeldahl method[30].

2.11 Statistical analysis

The variables were subjected to variance analyses (F test), following a factorial delineation in crossed model, in triplicate. The averages were compared by Tukey's multiple comparison method[38].

3 Results and discussion

Tables 1 to 11 show the results obtained for nickel, phosphorus, potassium, calcium, magnesium, sulfur, copper, iron, manganese and zinc content.

The potassium content remained constant in the different treatments (Table 2), despite the slightly higher content in pH 4.5 level. Possibly, the use of higher levels of nickel would lead to massive losses of potassium caused by heavy metal, as reported by another author[4].

The treatments with different concentrations of nickel interfered in the cell contents of calcium, copper and iron cell (Table 3, 5 and 8). The pH affected nickels toxicity, because the higher the pH the lower the intracellular calcium, copper and iron content.

The decrease in intracellular iron content indicates that nickel inhibited the ferrireductase enzyme responsible for iron absorption. This finding was previously reported in cellular treatment with cadmium[17]. As BLACKWELL and TOBIN[5] and ASSMANN et al.[4] reported for cadmium, nickel affected the intracellular magnesium and calcium contents. The accumulation of heavy metals in vacuoles probably caused displacement of the calcium and magnesium from their vacuolar storage sites, increasing the Ca^{+2} and Mg^{+2} concentrations in the cytosol. Since the plasmatic membrane is damaged by nickel, causing it to become fluidized, the transportation channels are opened, leading to a reduction of the intracellular level of free ions[4].

The cellular contents of manganese, zinc, nitrogen, sulfur and phosphorus did not vary in the different treatments (Tables 9, 10, 11, 7 and 6, respectively).

The pH influenced nickel absorption, since higher nickel absorption occurred in pH 3.5 than in pH 6.5 (Table 1). At higher levels of pH, nickel oxide and hydroxide are formed, decreasing the free ions available for interaction with the yeast[13] and thus reducing the absorption, which indicates toxicity.

Table 1. Nickel yeast content (mmol.kg $^{-1}$ in dw).

Treatment pHlevel	mmol.NiL^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	0.002^{Ea}	0.0598^{Da}	0.0498^{Da}	1.4310^{Ca}	3.6512^{Ba}	5.7877^{Aa}
4.5	0.002^{Ca}	0.0344^{Ba}	0.0420^{Ba}	0.0679^{Bb}	0.2131^{Ab}	0.5643^{Ab}
5.5	0.001^{Ca}	0.0523^{Ba}	0.0456^{Ba}	0.0767^{Bb}	0.2456^{Bb}	0.8765^{Ab}
6.5	0.002^{Ca}	0.0604^{Ba}	0.0382^{Ba}	0.0919^{Bb}	0.2787^{Ab}	0.2398^{Ab}
Standard derivation: 10.19%						

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

Table 2. Potassium yeast content (mol.kg $^{-1}$ in dw).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	0.3666	0.3922	0.3623	0.3581	0.3666	0.3922
4.5	0.4178	0.4369	0.4497	0.4455	0.4860	0.3964
5.5	0.3800	0.3368	0.3154	0.3261	0.3794	0.3730
6.5	0.3218	0.3282	0.3517	0.3538	0.3581	0.3304
Standard derivation: 17.67%						

According to the variance test (F test), the averages did not differ from each other at 1% of confidence.

Table 3. Calcium yeast content (mmol.kg $^{-1}$ in dw).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	27.86^{Aa}	26.61^{ABa}	25.87^{Ba}	23.70^{Ca}	$22.25^{C}d^{a}$	$21.83D^{a}$
4.5	22.87^{Ab}	19.65^{Bb}	18.50^{Bb}	17.26^{BCb}	17.26^{BCb}	$17.05D^{b}$
5.5	21.21^{Ab}	18.71^{Bb}	17.26^{Bb}	14.55^{BCc}	14.97^{Cc}	12.48^{Cc}
6.5	17.05^{Ac}	18.71^{Bb}	17.05^{Ab}	16.22^{Ab}	13.72^{Bc}	10.19^{Bc}
Standard derivation: 3.25%						

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

Table 4. Magnesium yeast content (mmol.kg $^{-1}$ in dw).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	56.91^{Aa}	52.80^{Ba}	53.83^{Ba}	51.09^{Ba}	50.40^{Ba}	46.29^{Ca}
4.5	52.80^{Ab}	50.40^{Ab}	51.77^{Ab}	50.06^{Aa}	42.46^{Bb}	45.26^{Ba}
5.5	52.46^{Ab}	48.34^{Bc}	47.32^{Bc}	44.91^{Cc}	46.29^{Bb}	$40.11D^{b}$
6.5	46.29^{Ac}	47.66^{Ac}	48.34^{Ac}	47.31^{Ab}	45.94^{Ab}	39.43^{Bb}
Standard derivation: 6.39%						

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

Table 5. Iron yeast content (mmol.kg $^{-1}$ in dw).

Treatment pH level	mmol.Ni L $^{-1}$					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	14.98^{Aa}	13.46^{Aa}	15.40^{Aa}	14.33^{Aa}	13.70^{Aa}	10.12^{Aa}
4.5	6.03^{Ab}	6.26^{Ab}	7.30^{Ab}	6.51^{Ab}	6.10^{Ab}	5.79^{Ab}
5.5	5.68^{Ab}	5.88^{Ab}	6.66^{Ab}	5.68^{Ab}	5.03^{Ab}	4.40^{Ab}
6.5	4.86^{Ab}	4.84^{Ab}	4.26^{Ab}	4.60^{Ab}	4.73^{Ab}	4.47^{Ab}
Standard derivation: 13.21%						

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

Table 6. Phosphorus yeast content (mol.kg $^{-1}$ in dw).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	0.3901	0.3794	0.3820	0.3886	0.3654	0.3390
4.5	0.3948	0.3417	0.3662	0.3605	0.3820	0.3874
5.5	0.3605	0.3901	0.3767	0.3632	0.3901	0.3901
6.5	0.3767	0.3901	0.3632	0.3901	0.3767	0.3632
Standard derivation: 5.96%						

According to the variance test (F test), the averages did not differ from each other at 1% of confidence.

Table 7. Sulfur yeast content (mmol.kg $^{-1}$ in dw).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	60.79	82.14	92.53	86.82	92.01	86.14
4.5	77.42	76.94	81.62	79.54	83.18	86.82
5.5	71.74	69.66	79.02	79.02	83.70	86.82
6.5	73.30	71.74	79.02	70.18	77.98	76.42
Standard derivation: 15.45%						

According to the variance test (F test), the averages did not differ from each other at 1% of confidence.

Table 8. Copper yeast content (mmol.kg $^{-1}$ in dw).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	0.4144^{Aa}	0.4196^{Aa}	0.4931^{Aa}	0.4983^{Aa}	0.5036^{Aa}	0.3987^{Aa}
4.5	0.1731^{Ab}	0.1416^{Ab}	0.1259^{Ab}	0.1259^{Ab}	0.1469^{Ab}	0.1416^{Ab}
5.5	0.1311^{Ab}	0.1626^{Ab}	0.1206^{Ab}	0.1206^{Ab}	0.1311^{Ab}	0.1154^{Ab}
6.5	0.1574^{Ab}	0.1574^{Ab}	0.1469^{Ab}	0.1574^{Ab}	0.1679^{Ab}	0.1469^{Ab}
Standard derivation: 15.98%						

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

Table 9. Manganese yeast content (mmol.kg $^{-1}$ in dw).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2,00	5,00
3.5	0.3017	0.3017	0.4389	0.4252	0.3977	0.3840
4.5	0.2880	0.2606	0.2469	0.2331	0.2743	0.2743
5.5	0.3154	0.2743	0.2469	0.2743	0.2880	0.2469
6.5	0.3154	0.3292	0.3429	0.2880	0.3566	0.2194
Standard derivation: 19.29%						

According to the variance test (F test), the averages did not differ from each other at 1% of confidence.

Table 10. Zinc yeast content (mmol.kg $^{-1}$ in dw).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	3.9354	3.8334	3.7671	3.5938	3.7213	3.4307
4.5	3.3950	3.2472	3.3033	3.3644	3.5479	3.4409
5.5	3.3186	3.3287	3.3491	3.2574	3.3899	3.3236
6.5	3.5989	3.6499	3.6601	3.5581	3.6856	3.3848
Standard derivation: 7.90%						

According to the variance test (F test), the averages did not differ from each other at 1% of confidence.

Table 11. Nitrogen yeast content (g.100 g^{-1} in dw).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	6.41	6.63	7.17	7.22	7.34	6.55
4.5	6.90	6.63	6.46	6.45	6.79	6.67
5.5	6.20	6.95	6.52	6.03	6.30	6.46
6.5	6.02	6.03	6.26	6.33	6.23	5.72
Standard derivation: 4.32%						

According to the variance test (F test), the averages did not differ from each other at 1% of confidence.

Tables 12, 13 and 14 show the results obtained for viability, budding rate and trehalose content. The viability rate and trehalose contents proved to be strongly correlated (Tables 12 and 14), indicating the decreasing viability as the pH decreased and the nickel content increased. The budding rate did not vary significantly in the different treatments (Table 13). GUTIERREZ et al.[12] reported results with a similar tendency as those shown here, differing only in the maintenance of cellular viability in pH 4.0 and contamination with 5.44 mmol of Ni L^{-1}. In addition, those authors reported a visible decrease in trehalose content, which is congruent with the data reported in this paper.

Trehalose is associated mainly with carbohydrate storage in fungus during the nonproliferation period[39]. LILLIE and PRINGLE[18] demonstrated that yeast survival depends on the stored trehalose level.

Figures 1 to 4 show CO_2 production in the different treatments. No difference was found in the fermentative velocity in the treatments with pH 5.5 and 6.5 (Figures 3 and 4). At pH 3.5 and 4.5, the fermentative velocity was lower in the treatments with nickel than for the control (Figure 1 and 2), showing a tendency to decrease as the metal concentration increased. This behavior may be attributed to the high availability of free nickel ions[13] and be correlated with the absorbed nickel content (Table 1), viability rate and trehalose content (Tables 12 and 14).

The slowest fermentation rate (Figures 3 and 4) and lowest viability rate (Figure 12) in the presence of nickel and low pH can be explained by the decrease in sugar absorption by yeast cells, due to the complexation of the phosphate groups of the plasmatic membrane by nickel, as proposed by VAN-STEVENINCK[42] and VAN-STEVENINCK e BOOIJ[43].

4 Conclusions

The *S. cerevisiae* yeast accumulated nickel at all the tested concentrations, since its accumulation potentially increases as the yeast is reused in subsequent fermentations. The pH affected nickel toxicity, which decreased as the pH increased.

Table 12. Viability rate (%).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	88.34^{Ab}	82.98^{Abb}	76.12^{Bb}	72.60^{Bc}	70.21^{Bd}	66.90^{Cd}
4.5	99.12^{Aa}	95.56^{ABa}	89.00^{Bb}	84.29^{BCb}	80.78^{Cc}	76.90^{Cc}
5.5	99.77^{Aa}	98.92^{Aa}	95.56^{ABa}	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 derivation: 19.60%						

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

Table 13. Budding rate (%).

Treatment pH level	mmol.Ni L^{-1}					
	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 derivation: 17.78%						

According to the variance test (F test), the averages did not differ from each other at 1% of confidence.

Table 14. Trehalose yeast content (%).

Treatment pH level	mmol.Ni L^{-1}					
	0.00	0.10	0.50	1.00	2.00	5.00
3.5	3.50^{Aa}	2.70^{Aa}	1.23^{Bc}	0.87^{Bc}	0.01^{Cb}	0.02^{Cb}
4.5	3.90^{Aa}	3.93^{Aa}	2.10^{Bb}	1.21^{Cb}	0.02^{Db}	0.01^{Db}
5.5	3.93^{Aa}	3.88^{Aa}	3.67^{Aa}	2.99^{Aa}	3.00^{Aa}	2.97^{Aa}
6.5	3.81^{Aa}	3.43^{Aa}	3.55^{Aa}	3.12^{Aa}	3.05^{Aa}	3.00^{Aa}
Standard derivation: 7.21%						

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

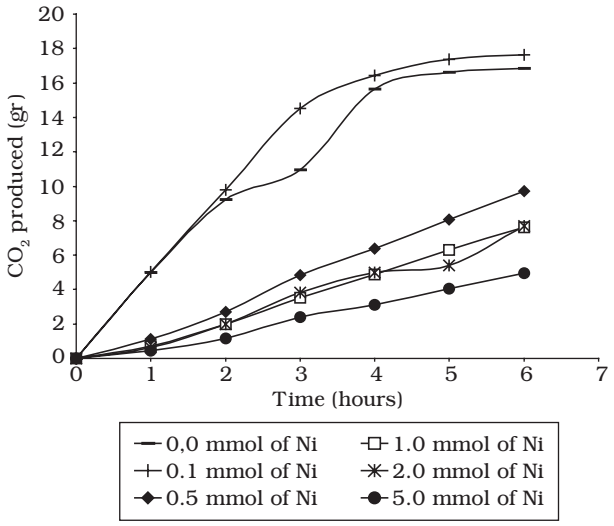

Figure 1. CO_2 produced in pH 3.5.

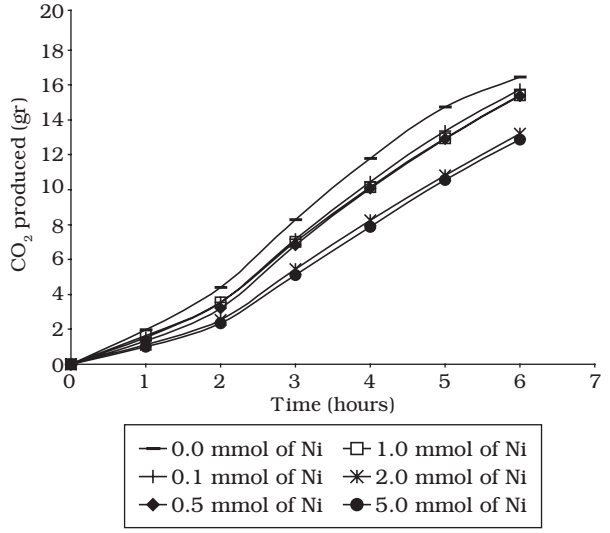

Figure 2. CO_2 produced in pH 4.5.

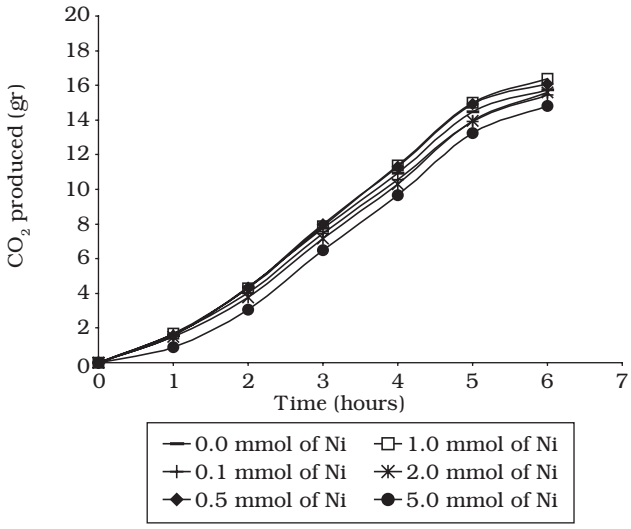

Figure 3. CO_2 produced in pH 5.5.

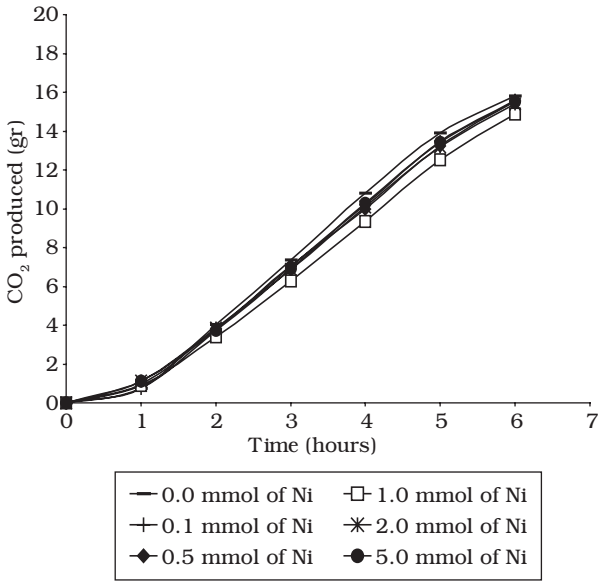

Figure 4. CO_2 produced in pH 6.5.

Nickel seems to influence the intracellular content of iron, magnesium and calcium, since the results obtained here were similar to those reported in the literature for other heavy metals. The trehalose values were strongly correlated with yeast viability and growth, since this parameter reflects the physiological stress to which the yeast is subjected.

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