DE-FARIA, F.; LEITE, C..;PRADO, R.; F.; MARQUES, T.; SILVA, S.; OLIVEIRA, S.; MARIANO-da-SILVA, S. Effect of pH, dextrose and yeast extract on cadmium toxicity about *Saccharomyces cerevisiae* PE-2. In: CONGRESSO DE PESQUISA, ENSINO E EXTENSÃO DA UFG - CONPEEX, 3., 2006, Goiânia. **Anais eletrônicos do XIV Seminário de Iniciação Cientifica** [CD-ROM], Goiânia: UFG, 2006. n.p.

EFFECT OF pH, DEXTROSE AND YEAST EXTRACT ON CADMIUM TOXICITY ABOUT *Saccharomyces cerevisiae* PE-2

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1 - INTRODUCTION

The ability of microorganisms to remove heavy metals from aqueous solution has long been of scientific interest. A variety of mechanisms are known for metal uptake, e.g. adsorption to cell wall, diffusion into the cells, and metabolism-dependent ion transport. These processes depend on many factors such as metal species and growth conditions of the organisms. However, very little information concerning resistance to toxic metals by yeast species has been published to date. Since previous studies indicated that the susceptibility to environmental toxicants can be influenced by medium characteristics (Hsu et al, 1992) we attempted to examine the effect of pH, yeast extract and dextrose contents on the cadmium toxicity towards *S. cerevisiae* PE-2 in order to optimize a bioassay with this organism.

2 - MATERIALS AND METHODS

2.1 - Yeast pre-growth

S. cerevisiae PE-2, caracterized by the karyotyping profile, was provided by the Biological Science Department Yeast Collection (ESALQ/USP). Yeast was reactivated in Yeast Extract Peptone Dextrose medium from a pure-culture (lyophilizated) and pre-grown at 30^{o}C, in sterelized molase medium with 6% of 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}).

2.2 - First growth assay

Fermentation was carried out with sterilized 75 mL of yeast extract 1% and dextrose 2% medium (YED) in 125 mL erlenmeyer flasks capped with aluminum foil with different cadmium concentrations (0.0 and 0.05 mmol L^{-1}) and different pH (2, 3, 4, 5, 6, 7 e 8). The pH of the suspensions was adjusted by using 0.1 mol L^{-1} H_2SO_4 or 0.1 mol NaOH. The flasks were inoculated in aseptic conditions with 1 mL of 1% (wet basis) yeast suspension and incubated at 30ºC, 70 RPM for 18 hours in orbital shaker. At specific times during fermentation (0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 hours), 1 mL portions of cell suspension were withdrawn 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. The experiments were made in triplicate.

2.3 - Second growth assay

Medium was prepared by dilution (full, half and quarter content to yeast extract and dextrose of 75 mL YED media concentration) and addition of 0.0 and 0.05 mmol L^{-1} of cadmium. The pH of the suspensions was adjusted to 5 by using 0.1 mmol L^{-1} H_2SO_4. The flasks were incubated at 30ºC, 70 RPM for 18 hours in orbital shaker. The experiments were made in triplication.

2.4 - Cell counting and yeast trehalose

After growth (18 hours) was made yeast viability and budding rate evaluation. Trehalose was determined by anthrone reaction according Brin (1966).

2.5 - Statistical analysis

Variance analysis (F-test) was used to analyze the variables, following a casual ship delineation in crossed model, with triplicate. The averages comparisons were made by multiple comparison Tukey method in a factorial delineation (Snedecor, 1967).

3 - RESULTS AND DISCUSSION

At low pH the yeast becomes more sensitive to the toxic effect (Table 1), and we accept pH between 3 and 6 as being optimal for metal/yeast interaction (Graphic 1 and 2). In this situation, the resulting chemical behavior of a given metal can be a complicated function of pH and medium matter (Bagy et al., 1991). Hsu et al. (1992), conclued that the dilution of YE medium (3 g yeast extract, 3 mg malt extract, 5 mg peptone and 10 g glucose in 1 liter distilled water) to ½ and ¼ with distilled water did not significantly alter the effect of cadmium on *S. cerevisiae*. In contrast, our results showed the high correlation of the growth medium dilution and the cadmium effects (Table 2). The S. cerevisiae PE-2 growth was significantly affected by 0.05 mmol L^{-1} of cadmium at 1 YE, and at ½ and ¼ YE the growth was totally inhibited. We observed that the susceptibility of S. cerevisiae PE-2 to cadmium was dramatically enhanced by the decrease of yeast extract strength. The cadmium ion has a strong affinity for organic materials, such as yeast extract (Ramamoorty & Kushner, 1975). Thus, there are two possible explanations for the toxicity decrease of cadmium when increasing yeast extract strength: the organic matter reacts with cadmium ions to form compounds that are less toxic than the ions themselves and/or the ions adsorbed on the surface of particles and are rendered less toxic (Bagy et al., 1991). The decrease of dextrose strength lowered the susceptibility of *S. cerevisiae* PE-2 to cadmium, probability because of the decrease of metabolic activity. It was found that the cadmium transport into the yeast cell (when the cadmium is more toxic) depends on energy, therefore glucose dependent (Rösick, 1986). Thus, in low dextrose, the cadmium was less toxic (*Table 2*).Yeast viability decreased in parallel with trehalose content, apparently in response to cadmium toxicity. Therefore, trehalose concentrations may be an important indicator of cadmium stress on yeast.

Table 1. Trehalose content, viability and building rate in first yeas assay

Treatment	Trehalose (g $100g^{-1}$)	Viability (%)	Building (%)
Initial	5.85 a	95.91 a	25.61 a
pH 2.0 - 0.0 mmol L^{-1} Cd	0.00 e	7.35 d	0.00 c
pH 3.0 - 0.0 mmol L^{-1} Cd	3.09 c	63.88 c	24.93 a
pH 4.0 - 0.0 mmol L^{-1} Cd	4.87 b	98.53 a	23.64 a
pH 5.0 - 0.0 mmol L^{-1} Cd	4.84 b	99.50 a	20.03 ab
pH 6.0 - 0.0 mmol L^{-1} Cd	4.77 b	99.13 a	24.57 a
pH 7.0 - 0.0 mmol L^{-1} Cd	4.84 b	99.27 a	23.78 a
pH 8.0 - 0.0 mmol L^{-1} Cd	4.87 b	99.54 a	23.63 ab
pH 2.0 - 0.05 mmol L^{-1} Cd	0.00 e	0.00 e	0.00 c
pH 3.0 - 0.05 mmol L^{-1} Cd	0.10 e	0.00 e	0.00 c
pH 4.0 - 0.05 mmol L^{-1} Cd	0.99 d	62.26 c	15.01 b
pH 5.0 - 0.05 mmol L^{-1} Cd	1.45 d	85.49 b	23.10 ab
pH 6.0 - 0.05 mmol L^{-1} Cd	3.19 c	90.30 b	27.55 a
pH 7.0 - 0.05 mmol L^{-1} Cd	4.87 b	98.14 a	27.39 a
pH 8.0 - 0.05 mmol L^{-1} Cd	4.97 b	98.72 a	25.10 a
Coefficient of Variation (%)	5.986	2.056	12.09

The averages followed by the same letters don't differ among themselves, according to the test of Tukey to 1% of confidence

Table 2. Yeast biomass, viability, building rate and trehalose content in second yeast assay (1YE=full; ½YE=half; ¼YE=quarter) and dextrose (1D=full; ½D=half; ¼D=quarter)

treatment	Yeast (g 100 mL^{-1})	Viability (%)	Building (%)	Trehalose (g 100 g^{-1})
initial	-	99.78a	25.66a	5.23a
1YE + 1D - 0.0 mmol L^{-1} Cd	1.6750a	99.51a	26.13a	4.51a
1YE + ½D - 0.0 mmol L^{-1} Cd	1.6409a	99.60a	22.10a	6.22a
1YE + ¼D - 0.0 mmol L^{-1} Cd	1.5768a	99.63a	11.54b	6.28a
½YE + 1D - 0.0 mmol L^{-1} Cd	1.5077a	98.69a	19.51a	1.60b
½YE + ½D - 0.0 mmol L^{-1} Cd	1.4534a	98.63a	10.97b	5.22a
½YE + ¼D - 0.0 mmol L^{-1} Cd	1.4972a	99.00a	15.91ab	6.07a
¼YE + 1D - 0.0 mmol L^{-1} Cd	1.5728a	84.02b	19.93a	0.19c
¼YE + ½D - 0.0 mmol L^{-1} Cd	0.6872b	92.22a	11.73b	0.03c
¼YE + ¼D - 0.0 mmol L^{-1} Cd	0.5243b	96.70a	19.33a	0.01c
1YE + 1D - 0.05 mmol L^{-1} Cd	0.7722b	56.12b	12.09b	0.91b
1YE + ½D - 0.05 mmol L^{-1} Cd	0.7662b	45.89b	26.12a	1.16b
1YE + ¼D - 0.05 mmol L^{-1} Cd	0.8085b	44.12b	28.22a	2.54a
½YE + 1D - 0.05 mmol L^{-1} Cd	0.0062c	0.00c	0.00c	0.02c
½YE + ½D - 0.05 mmol L^{-1} Cd	0.0074c	0.00c	0.00c	0.01c
½YE + ¼D - 0.05 mmol L^{-1} Cd	0.0065c	0.00c	0.00c	0.03c
¼YE + 1D - 0.05 mmol L^{-1} Cd	0.0097c	0.00c	0.00c	0.03c
¼YE + ½D - 0.05 mmol L^{-1} Cd	0.0073c	0.00c	0.00c	0.03c
¼YE + ¼D - 0.05 mmol L^{-1} Cd	0.0066c	0.00c	0.00c	0,01c
Coefficient of Variation (%)	9.98	9.05	19.90	12.31

The averages followed by the same letters don't differ among themselves, according to the test of Tukey to 1% of confidence

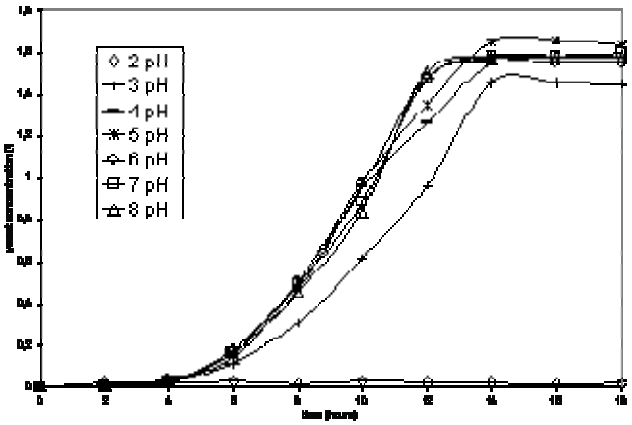

Figure 1. Effects of pH on the growth of *S. cerevisiae* PE-2 in medium without cadmium

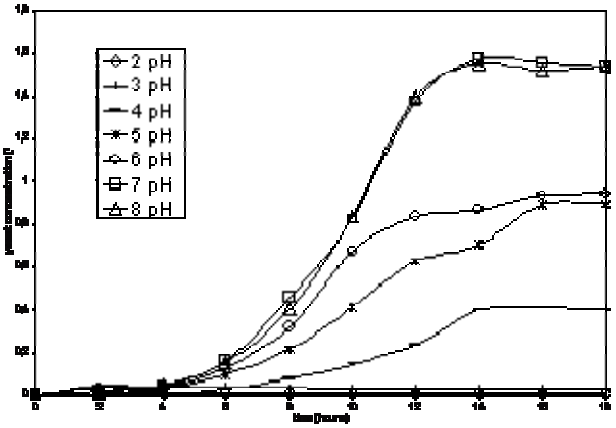

Figure 2. Effects of pH on the growth of S. cerevisiae PE-2 in medium with 0.05 mmol L^{-1} of cadmium.

4 - CONCLUSIONS

The toxicity of cadmium to *Saccharomyces cerevisiae* PE-2 is apparently dependent of the chemical characteristics of the growth medium. The toxicity of a toxicant my be reduced by some of the specific properties of one growth medium whereas in another with different chemical characteristics, the toxicity of an equivalent dose of some toxicant may be potentiated. Thus, to success in toxicity studies, the influence of the chemical factors on toxicant toxicity should be considered.

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