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NEUTRON ACTIVATION ANALYSIS OF CADMIUM BIOREMEDIATION BY YEAST ISOLATED FROM THE FERMENTATION OF CACHAÇA

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ABSTRACT

The accumulation of heavy metal in urban environment is a final result of industrial waste discharges. The removal and recovery of heavy metals from contaminated water and wastewater is important in the protection of the environment and human health. There are several chemical technologies used to remove heavy metals. Most of these are ineffective or excessively expensive when the metal concentrations are less than 100 mgL^{-1}. Biological treatment with bioremediation, is an innovative technology available for heavy metal polluted wastewaters. Brazil has a big production of yeast as a by-product of the fermentation of sugar cane for the production of ethanol or, for the production of artisanal *cachaça*, notedly in the state of Minas Gerais. Biological organisms remove metals through of two processes: bioaccumulation and biosorption. This research used neutron activation technique to determine the capacity of 10 isolated yeast of the fermentation for the withdrawal of cadmium. The efflux of ions K^+, was also analyzed by the same technique after the incorporation of cadmium by cells. This work showed that the neutron activation analysis is a suitable technique to quantification the metal absorbed from liquid solution and that isolated strains of the fermentation of cachaça are more efficient in removing cadmium of the liquid solution that the laboratorial strain. The influences of the metals on the growth of the cells are also observed. The results obtained were compared with the yeast strain of laboratory, *Saccharomyces cerevisiae* W303-WT. The tolerance of cadmium to concentration of 100 mgL^{-1} was evaluated.

1. INTRODUCTION

The intensification of the industrial activities in the last decades is the great contributor for the increase of heavy metals in the environment, mainly in liquid solutions [1]. Conventional methods such as chemical precipitation and adsorption by activated carbon present disadvantages in the treatment of effluent that include the incomplete removal of the metal, expensive reagents and equipments and high energy cost and production of toxic waste [1] [2] [3].

The bioremediation is a technology for removal and/or heavy metal recovery, of contaminated areas. Bioremediation can be defined as "a process where if it uses solids of vegetal origin or, microorganisms, in the retention, removal or, metal recovery heavy of a

liquid environment" [4]. The bioremediation has received great attention, as much as scientific newness as also for the possible application in the industry. The microorganisms including bacteria, algae, fungi and yeast are efficient in bioremediation. The conventional processes of diluted metal removal in effluent (concentrations below of 100 mgL^{-1}), are not so efficient and present high costs.

The success of bioremediation process depends on the disponibililitty of biomass. This biomass can be cheaply and easily supplied in rather substantial quantities, as a by-product from the established industrial fermentation processes. That constraint make our choose by *Saccharomyces cerevisiae* cells of some strains isolated in the region. It is known by all that Brazil is a great productor of the yeast *Saccharomyces cerevisiae*. Theses cells are a by-product of sugar cane fermentation used on the generation of ethanol as a fuel. Also *Saccharomyces cerevisiae* is used in the beverage industries of cachaça. The production of artisanal cachaça at Minas Gerais State is important economic activity into the region.

Cadmium is an important environmental heavy metal. Occupational and environmental pollution with cadmium results mainly from mining, metallurgy industry and manufactures of nickel-cadmium batteries, pigments and plastic stabilizers. It has been classified as a carcinogen by the World-wide Organization of Health and Agency for Research on Cancer. Cadmium affects cellular DNA and enhances oxidative stress. However, the production of reactive species of oxygen (ROS) is indirect. Cadmium is a not redox-active metal and cannot itself direct Fenton type-reaction. Cadmium induces oxidative stress arising from indirect processes, a decrease of cellular antioxidantes and exhalation of ROS by mitochondria [5].

The goal of this work was test and compares the capacity of *Saccharomyces cerevisiae* cells into incorporate Cd^{2+} on liquid medium. Two processes were used for the incorporation of the metal: bioaccumulation (use of living cells) and biosorption (use of dead cells). The results obtained were compared with the yeast strain of laboratory, *S. cerevisiae* W303-WT. It was used the neutron activation technique to determined the concentration of the metal incorporated by the cells. The neutron activation was an easy, rapid and suitable technique to do these metal determinations on yeast cells. The influences of the metals on the growth of the cells are also observed.

2. MATERIALS AND METHODS

2.1. Microorganisms and Growth Conditions

The strains used in this study are listed in table 1. The composition of the culture medium used were 2% glucose, 2% peptone, 1% yeast extract (medium YPG), when necessary 2% agar was additioned. Stock solution of $CdCl_2$ were made on water, sterilized separately and added to the liquid medium after autoclaving in accordance with the necessity of each experimental protocol.

Table 1. Strain used

Yeast strains	Destilleries	City	Region
S. cerevisiae UFMGA 829	Velha Aroeira	Viçosa	Zona da Mata
S. cerevisiae UFMGA 905	Germana	Nova União	Zona Metalúrgica
S. cerevisiae UFMGA 1003	Seleta Boazinha	Salinas	Jequitinhonha
S. cerevisiae UFMGA 1007	Seleta Boazinha	Salinas	Jequitinhonha
S. cerevisiae UFMGA 1011	Seleta Boazinha	Salinas	Jequitinhonha
S. cerevisiae UFMGA 1386	Brumado Velho	Brumado Velho	Zona Metalúrgica
S. cerevisiae UFMGA 2097	Seleta Boazinha	Salinas	Jequitinhonha
S. cerevisiae UFMGA 2255	Rainha do Vale	Moeda	Zona Metalúrgica
S. cerevisiae UFMGA 2382	Gotas de Minas	Ouro Preto	Zona Metalúrgica
S. cerevisiae UFMGA 2464	Seleta Boazinha	Salinas	Jequitinhonha
S. cerevisiae W303-1A (WT)	Laboratorial	-	-

2.2. Incorporation of Cadmium

2.2.1. Bioaccumulation

Yeast strains were grown in YPG medium during 24 hours, collected by centrifuged (10 min 3000 rpm) and ressuspended in new medium. After 4 hour the cells were collected by centrifugation transferred to 30 mL of YPG containing 100 mgL^{-1} of cadmium chloride. After 24 hours the solution was filtered and immediately washed with cold water. The pellet was transferred to appropriate tubes and dried at 70 ^{o}C during 3 days.

2.2.2. Biosorption

Yeast strains were grown in YPG medium during 24 hours, collected by centrifuged (10 min 3000 rpm) and ressuspended in new medium. After 4 hour the cells were killed by autoclavation, collected by centrifugation transferred to 30 mL of YPG containing 100 mgL^{-1} of cadmium chloride. After 24 hours the solution was filtered and washed immediately with cold water. The pellet was transferred to appropriate tubes and dried at 70 ^{o}C during 3 days.

2.3. Determination Intracellular of Cadmium and Potassium

The neutron activation analysis, k_0-method, was applied for determining the Cd and K concentrations in the same samples. Cells were weighted in the irradiation vials and, accompanied by standards of Cd and K, it was irradiated during 8 h in the TRIGA MARK I IPR-R1 research nuclear reactor, located at CDTN/CNEN, that at 100 kW the average thermal neutron flux is $6.6x10^{11}$ $n.cm^{-2}$ s^{-1}. The gamma spectroscopy was performed on an HPGe detector with 15% efficiency and the peak characteristic of each radionuclide were used to calculate elemental concentration (Cd and K) in accordance with the methodology [6].

2.4. Cellular Tolerance to Cadmium

Cell tolerance to cadmium was determined based on growth on YPG agar medium with 100 mgL^{-1} of cadmium chloride. Cells were submitted to serial dilutions. The plates were incubated at 30ºC up to 3 days and the plates were photographed.

2.5. Statistical Analysis

The results were expressed as mean and ± standard deviation of three different experiments. Statistical difference of results was performed using Student's test t and One-Way ANOVA with levels of significance of $P < 0.05$.

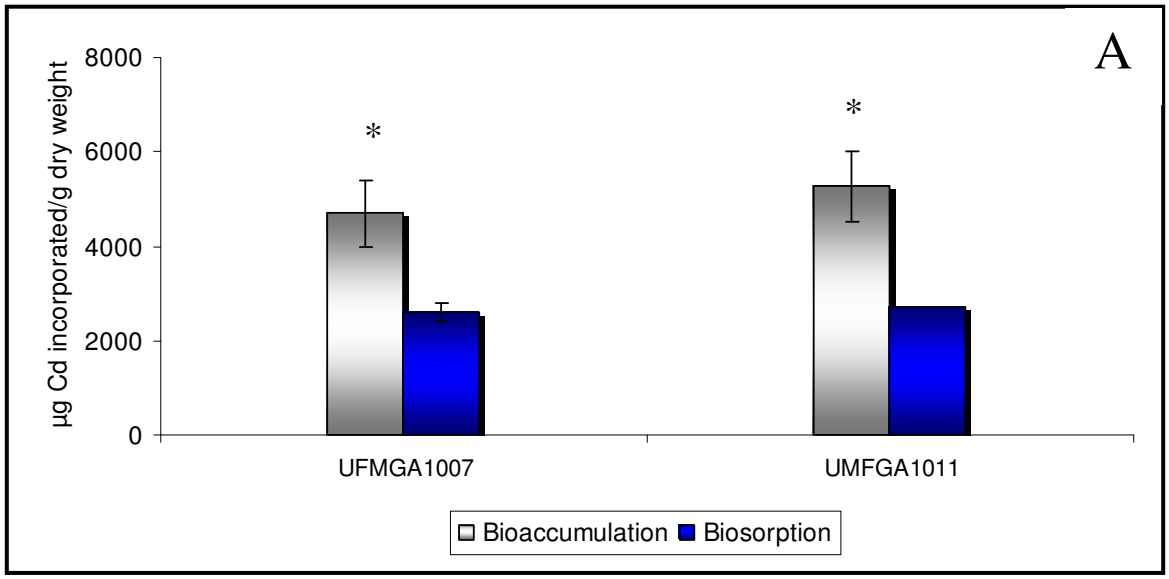
3. RESULTS AND DISCUSSION

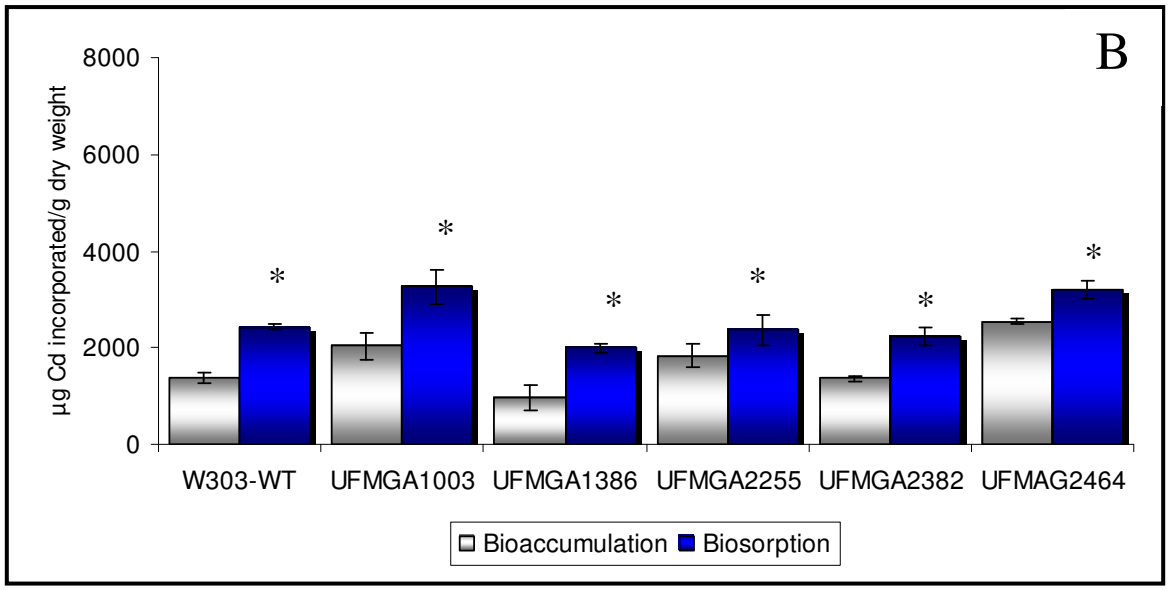
3.1. Determination of Cadmium Incorporation by Viable and Non-viable Cells

When comparing processes of bioaccumulation and biosorption (Fig. 1), it appears that cells incorporate different amounts of metal as process tested. Yeasts UFMG-A1011 and UFMG-A1007 incorporate significantly more cadmium when live, while strains, W303-WT, UFMG-A1003, UFMG-A1386, UFMG-A2255, UFMG-A2382 and UFMG-A2464 incorporated more cadmium when dead. Yeasts strains, UFMG-A 829, UFMG-A905 and UFMG-A2097 remove the same quantity of the metal by viable and not viable cells. The biosorptive capacity of dead cells may be greater, equivalent to or less than that of living cells [4].

The results presented show important aspects of bioremediation of cadmium by *S. cerevisiae*, suggesting the dead biomass as material more suitable for application in practice, giving greater capacity for incorporation of metal (results obtained in six of the eleven tested yeast) in absence of cell sensitivity, thus excluding any interference in the metabolic and physiological process. However cells from UFMG-A1007 and UFMG-A1011 incorporated

respectively 80% and 95% more cadmium, should be selected for the process, due to the significant uptake of metal and the large difference in incorporation in relation to dead biomass. These two strains were most efficient in the removal of metal in liquid medium.





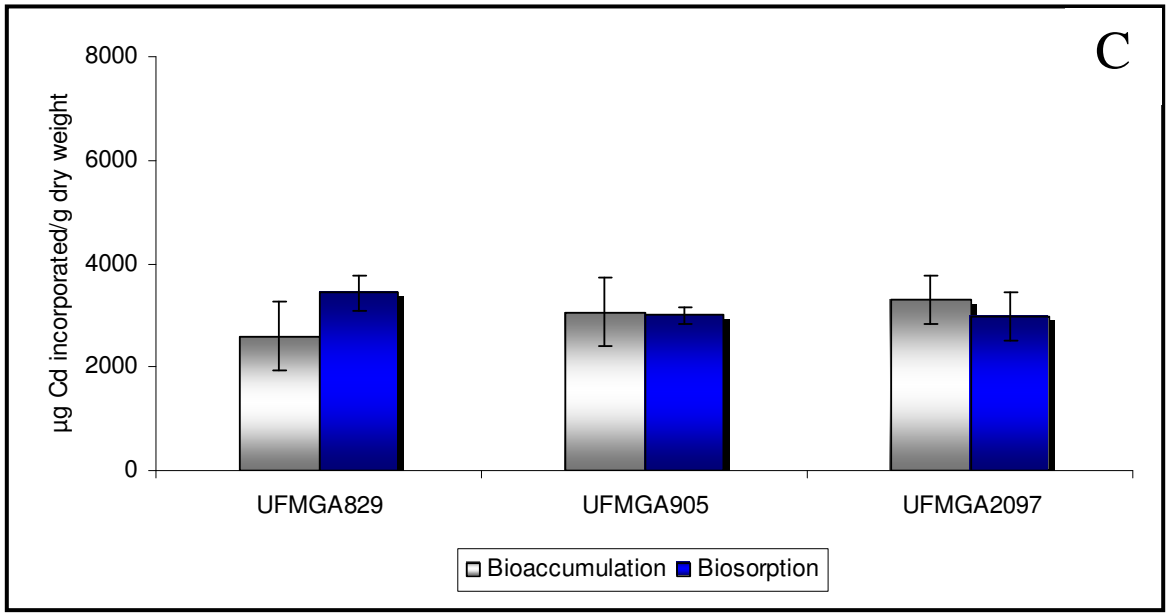


Figure 1. Determination of cadmium incorporated by viable cells and non-viable in 24h. Bioaccumulation > Biosorption (A), Bioaccumulation < Biosorption (B) and Bioaccumulation = Biosorption (C), *P<0,05.

3.2. Determination of Efflux of Potassium Ions After Incorporation of Cadmium

Analyzing results presented in table 2, it is observed that efflux of potassium ions is related to incorporation of cadmium. The strains (W303-WT, UFMG-A829, UFMG-A905, UFMG-A1003, UFMG-A1007, UFMG-A1011, UFMG-A1386, UFMG-A2097, UFMG-A2382 and UFMG-A2464) lost K^+ significantly in presence of cadmium. But this loss was not proportional to amount of cadmium incorporated. This can be explained by differences in the synthesis of ion channels and transporters of the yeast. Exposure of *S. cerevisiae* to toxic concentrations of Cd^{2+} generally results in permeabilization of the plasma membrane, which is readily detected with a fast and non-stoichiometric loss of cellular K^+ [7]. Among strain studied, laboratorial strain showed higher efflux of potassium, proving be more susceptible to loss of homeostasis after exposure to Cd^{2+}. The yeast UFMG-A2255 showed no difference amount of potassium in control and in cells exposed to cadmium. Studies reported large losses of the ion (K^+) cells by *S. cerevisiae* exposed to 50 and 100µM of $Cd(NO_3)_2$ [7].

Table 2. Amont of potassium in control cells and exposed to 100 mg.L^{-1} of cadmium chloride

Strain of *S. cerevisiae*	**Control** (µg K^+/g dry weight)	**Cells exposed to cadmium** (µg K^+/g dry weight)
W303-WT	18067[(a)]±1069[(b)]	11733±58[c]
UFMG-A 829	18800±173	15400±2227[c]
UFMG-A 905	17000±265	14400±265[c]
UFMG-A 1003	19800±173	15233±586[c]
UFMG-A 1007	19550±404	17333±321[c]
UFMG-A 1011	19467±289	17767±1650[c]
UFMG-A 1386	11633±153	7900±1732[c]
UFMG-A 2097	15733±635	12867±808[c]
UFMG-A 2255	13733±603	13767±451
UFMG-A 2382	18367±751	15300±173[c]
UFMG-A 2464	17700±400	13000±1825[c]

a – mean experiment in triplicate
b – standard deviation
c – significant difference on the control (P<0,05)

3.3. Metal Tolerance

It is observed in Fig. 2, formation of colonies in all strains in control situation, whereas plates containing 100 mgL^{-1} of $CdCl_2$ cell growth was inhibited or absent. Occurred cell growth of all strains exposed to cadmium in 10^8 cells/mL concentration, whereas lower concentrations only cells isolated from fermentation of "cachaça" grown. Strain UFMG-A905 was the only strain that form colonies at the concentration of 10^6 cells/mL. No cell growth was evident in lower dilutions. Yeast strains of *S. cerevisiae*, showing that cells were inhibited or less grown on solid medium supplemented with cadmium [8].

The result shows that the yeasts from the fermentation are more tolerant to effects of cadmium than laboratory strain, which is an advantage in process of bioremediation.

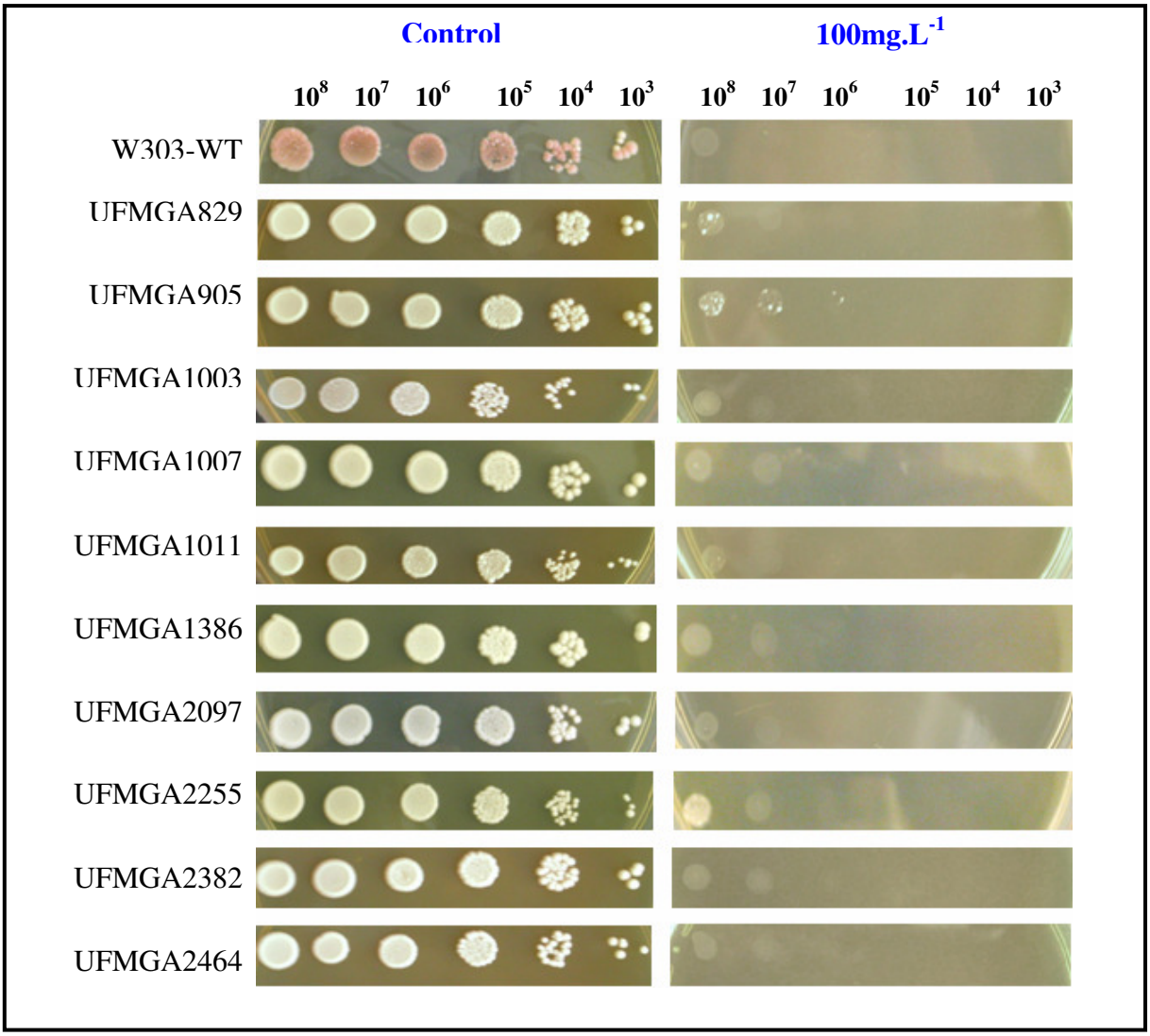


Figure 2. Determination of cell survival in solid medium suplplemented with cadmium chloride.

4. CONCLUSION

- This work showed that the neutron activation analysis is a suitable technique to quantification the metal absorbed from liquid solution.
- The living cells of UFMGA1011 and UFMGA1007, are more efficient in removing cadmium by bioaccumulation;
- The yeast strains UFMGA829, UFMGA905 and UFMGA1003 incorporate more metal, when compared with the laboratory strain, in the process of biosorption;
- Yeast cells can remove cadmium at concentrations of 100 $mg.L^{-1}$. This is a very important result because it is important remember the high cost of conventional chemical methods when employed in diluted liquid solutions.

- The laboratory strain showed largest efflux of potassium is more sensitive to loss of homeostasis after exposure to cadmium;

- Yeasts from the fermentation are more tolerant to effects of cadmium than laboratory strain.

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