FUNCTIONING OF DIVALENT ALKALINE METAL ON YEAST MULTIPLICATION IN HEAVY METAL CONTAMINATED SOIL

Hosam E.A.F. BAYOUMI HAMUDA[1], Nikolett TÓTH[2]

[1]Óbuda University, Rejtő Sándor Faculty of Light Industry and Environmental Protection Engineering,
Environmental Protection Engineering Institute
H-1034 Budapest Doberdó Str. 6.
[2]Szent István University, Postgraduate School for Environmental Sciences
H-2103 Gödöllő Páter K. Str. 1., e-mail: hosameaf@gmail.com.

Summary: Microbial parameters appear to be very useful in monitoring soil contamination by heavy metals. The toxic heavy metals cause serious threat to the environment, Nowadays, the most an important ecological problem is to correct the toxic effect of heavy metals in contaminated soils. *In vitro*, two strains of *Saccharomyces cerevisiae* (NSS5099 and NSS7002) were tested in order to investigate their tolerance to heavy metals. The growth kinetics of two yeast strains in contaminated growth media by Cu^{2+}, Pb^{2+}, Cd^{2+} and Ni^{2+} were studied at 50 µM. The toxicity decreasing order of the investigated heavy metal salts on tested yeast strains was found to be $Cu^{2+} > Pb^{2+} > Cd^{2+} > Ni^{2+}$. Ions of Cu^{2+}, Pb^{2+} and Cd^{2+} at 350 µM and Ni^{2+} at 450 µM induced a decrease in the number of viable cells by 50% after 48 h incubation at 25°C. The addition of 50 mM $Ca(HCO_3)_2$, 75 mM $MgSO_4$, or 150 mM K_2SO_4 in the growth broth medium before addition of 350 µM Cu^{2+}, Pb^{2+} and Cd^{2+} or 450 µM Ni^{2+} shows a protective action against cell death and decreased the toxicity effect. The addition of alkaline metals reduced the effect of 350 and 450 µM of all investigated metals by 40%. The results obtained in the presented study exhibit the higher potentiality of *S. cerevisiae* strain NSS7002 than the strain NSS5099 to be used for decontamination of soil containing heavy metal ions. Further task is going to examine the range of metal bioaccumulation in the yeast cells and the ability of these strains to be environmental bioremediators.

Introduction

One of the main pollutants of the global pollution is the toxic heavy metals. Since it is impossible to degrade those pollutants by any means, the only way to remove them from the environment is to exclude metals from cycling through their concentration, with a possible recovery and reuse. This would also reduce the consumption of non-renewable resources (CHOJNACKA 2010). All organisms must possess mechanisms that regulate metal ion accumulation and thus, avoid heavy metal toxicity. Several resistance mechanisms exist to lessen or prevent metal toxicity. These include resistance to metals that are always toxic to the cell and serve no beneficial function, such as cadmium (Cd), lead (Pb) and mercury (Hg), and also include resistance to metals such as copper (Cu), iron (Fe), nickel (Ni), chromium (Cr) and zinc (Zn), which are toxic at high concentrations but are absolutely essential in trace amounts. The phenomenon of biosorption is defined as a metabolism independent adsorption of pollutants based on the partition process on a microbial biomass, or it is a passive non-metabolically-mediated process of metal binding by biosorbent. Microorganisms such as bacteria, yeasts, fungi and algae have been used as biosorbents of heavy metals. Among these, yeasts are known to be selective metal biosorbents as compared to fungi, actinomycetes and bacteria (ZOUBOULIS et al. 2001). Some metals such as calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) are essential for microbial life, other metals for example, Cu, Fe, manganese (Mn), Ni and Zn are required in trace concentrations (micronutrients) and some metals (e.g., Cd and

Pb) that are considered to be pollutants, as they are not necessary for biological functions and are toxic. *Saccharomyces* play a significant role in agricultural industry such as brewing, wine-growing, and other distillation, but also have a very important role in pharmaceutical industry and as a fodder (Horváth 1970). Investigations conducted by several researchers demonstrated that *S. cerevisiae* is capable of accumulating heavy metals such as Cu, Cd, Pb, Zn, Cr and Ni ions (Engl and Kunz 1995; Volesky and Phillips 1995). One major mechanism of Cu toxicity towards microorganisms is disruption of plasma membrane integrity. The influence of plasma membrane fatty acid composition on the susceptibility of *S. cerevisiae* to Cu^{2+} toxicity was investigated (Avery et al. 1996).

Collins and Stotzky (1992) suggested that the toxicity of Cd, Cr, Cu, Hg, Ni, Pb, and Zn, and of Na and Mg to microorganisms varies with pH because the hydrolyzed speciation forms of these metals, which occur at higher pH values, bind on the cell surface and alter the net charge of the cell. This change in charge could affect various physiological functions of the cell, as well as its interactions with other cells and inanimate particulates in the environment. The yeast *S. cerevisiae* as a promising biosorbent has been used to remove Cr^{4+}, Fe^{3+} (Goyal et al. 2003), Cd^{2+} (Liu et al. 1997), Cu^{2+} (Jianlong 2002 and Machado et al. 2009) from aqueous solutions.

Removal of polluting metals from contaminated environments is often difficult and remediation through common physico-chemical techniques is highly expensive and unsuitable in case of voluminous effluents containing complexing organic matter and low metal contamination. Biotechnological approaches may be an alternative remediation choice, and the increase of environment pollution by heavy metals turned the interest to live organisms that are tolerant to these metals. Biosorption (passive uptake) is one of the mechanisms of microorganisms resistance to heavy metals and yeasts as biosorbents are of special interest (Wang and Chen 2009, Saleem et al. 2008, Can and Jianlong 2007, Bender and Phillips 2004, Malik 2004, Akhtar et al. 2004, Lloyd et al. 2003). The most well-known and commercially significant yeasts are the related species and strains of *S. cerevisiae*, with high potential as bioremediation effectors (Naeem et al. 2006, Machado et al. 2008, 2009). Most bioremediation studies using *S. cerevisiae* involve the excellent biosorption capacity of yeast cells (Wang and Chen 2006, 2009).

Interest has arisen because of the biotechnological potential of microorganisms for metal removal and/or recovery, the possible transfer of accumulated metals to higher organisms in food chains, and the toxicity of heavy metals towards microbial metabolism and growth. Toxic effects are generally related to the strong coordinating abilities of heavy metals, and they include blocking of functional groups and conformational modification of cellular macromolecules, displacement of essential ions, and disruption of cellular and organellar membrane integrity (Gadd 1993).

The study of the interactions between metals and fungi has long been of scientific interest. In an environmental context, accelerating pollution by toxic metals, metalloids and radionuclides has influenced research towards the biotechnological potential of utilizing microorganisms for metal removal and/or recovery from the biosphere.

Yeasts possess a potential for accumulating a range of metal cations. Tolerance and uptake of heavy metals by microorganisms has received much attention because of their potential application in bioremediation and biotreatment systems. *Saccharomyces cerevisiae* biomass plays an important role in the investigations in the field of heavy metal biosorption. It can be obtained in great quantities as waste products of many fermentation

processes. Besides, it can grow in solutions with high concentrations of heavy metals. It can accumulate metal ions in biomass (VOLESKY and PHILLIPS 1995). The intensification of industrial activity during the recent decades is greatly contributing to the increase of heavy metals in the environment. Humanity and the environment have to face up to the fact of considerable environmental pollution. There are many reviews on algae, bacteria, fungi or higher plants that remove and/or accumulate large amounts of heavy metals from their external environment. The main reaction for heavy metal to be combined with the microbial surface can be described as an ion-exchange reaction between heavy metal and Ca^{2+}, Mg^{2+} or K^+ in the cell wall. This reaction is also observed in mold (KAPOOR and VIRARAGHAVAN 1997), and yeast (SINGLETON and SIMMONS 1996). Temperature, pH, the number of yeast cells and their physiological activity as well as the presence of other ions in the medium may exert a significant effect on the dynamics of Mg biosorption by cells (TUSZYNSKI and PASTERNAKIEWICZ 2000; PARK et al. 2003).

The heavy metal removal mechanism of microorganisms can be classified as extra-cellular accumulation/precipitation, cell surface sorption/precipitation, or intracellular accumulation, according to the location of the biosorption of the metal removal from solution (VEGLIO and BEOLCHINI 1997). In the concept of biosorption, several physical or chemical processes may be involved such as physical and/or chemical adsorption, ion exchange, coordination, complexation, chelation, and micro-precipitation. Biomass cell walls, consisting mainly of polysaccharides, proteins, and lipids offer many functional groups, which can bind metal ions such as carboxylate, hydroxyl, sulphate, phosphate, and amino groups.

Yeasts, or any other microorganisms, contains many potential sites for the sorption of metal ions, and it is unlikely that only one type of molecule or functional group would be responsible for the absorption of the metal ions. The mechanism of metal ions accumulation in yeast is well known to be composed of two or three steps (SINGLETON and SIMMONS 1996; SUH et al. 1998). The first is a rapid binding to negatively charged groups on the cell surface and a passive transport of metal ions through the cell wall for short time within 3.5 min. The second is the penetration through the cell membrane and into the cytoplasm. The third is the metal ions accumulation in the cell cytoplasm. One of the most serious forms of the environmental damages is accumulation of heavy metals. Heavy metal contamination exposes flora, fauna, and indirectly humanity to danger because of the difficulty of their breakdown and their accumulation in the environment. Microorganisms are more sensitive to heavy metal contamination than those plants and animals, which are present in the same soil (GILLER et al. 1997). Generally, it has been reported that fungi are more tolerant to heavy metals than bacteria and actinomycetes (HIROKI et al. 1992).

The surface of a cell has an important role in the relationship between the cell and its environment, as the surface is in direct contact with the ambient environment of the cell, and both essential and nonessential metal ions are transported across the surface into the cell. When heavy metals are deposited into an environment, they may bind on the surface of microorganisms, which is probably the initial step in the uptake and concentration of the metals by the microbes and in the toxic effects of these metals. The cell surface is important in microbial ecology, in the adhesion of microbes on surfaces, and in interactions between microorganisms and external environment. Natural binding of metals with yeast cells has a character of chemisorption that has been described by the Langmuir's equation (LO et al. 1999). The Ca^{2+} or Mg^{2+} ions are first bound with the cell wall, and

primarily with negatively charged functional groups present in its structure, and thereafter they can be transferred to the cell's interior (Saltukoglu and Slaughter 1983). The aim of the present investigation is to examine the tolerance of two *S. cerevisiae* strains to the toxicity of Cu^{2+}, Cd^{2+}, Pb^{2+} and Ni^{2+}. Secondly, to study the functioning of divalent Ca^{2+} and Mg^{2+} metals in comparison with K^+ to correct the negative impacts of heavy metals on yeast growth and multiplication.

Materials and Methods

Maintenance and cultivation

Two strains of *S. cerevisiae* (NSS5099 and NSS7002) were isolated from the rhizosphere of maize (*Zea mays* L.) grown in „kovárvány" brown forest soil (obtained from the Research Centre of Agricultural Science Centre, University of Debrecen, Nyíregyháza) amended with 50% of municipal sewage sludge in 1999. The strains were grown and maintained at 4°C in a medium containing (gl^{-1}): yeast extract 10, peptone 10, dextrose 20, and agar 20 (YPDA). The pH of the medium was adjusted to 5.2. The medium was autoclaved at 121°C for 15 min. The cells were grown in YPD medium at 30°C for 2 days in shaking incubator at 150 rpm. In a test tube containing fresh yeast culture on YPDA, the biomass was washed out with physiological solution (0.85% NaCl). Yeast cultures were grown aerobically.

Under sterile conditions, a 40 ml YPD broth medium placed in a 100 ml Erlenmeyer flask and 5 ml yeast cell suspension (up to $5x10^6$ cells ml^{-1}) is added to obtain suspension, which contained 5 mg of yeast l^{-1}. The cultivation was carried out on an orbital shaker at 150 rpm and 28°C. To examine the effect of the heavy metal ions on the growth of the two strains of *S. cerevisiae*, Cu^{2+}, Cd^{2+}, Pb^{2+} and Ni^{2+} ions of $CuSO_4$, $Cd(NO_3)_2$, $Pb(NO_3)_2$ or $NiSO_4$, respectively, were added to the nutritional medium at different concentrations. The solution of metal salts was sterilized by biological membrane filter of size pore 45 μm.

Kinetic study of cell growth in different heavy metal

The effect of the above mentioned metals at 50 μM concentration was examined after 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66 and 72 hours on cell growth kinetics at 28°C. The incubation was carried out in orbital shaker with 150 rpm.

Determination of cell growth in different heavy metal concentrations

The yeast cell growth was studied in YPD broth medium that contained (0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 μM) concentration of Cu, Cd, Pb and Ni salts at 28°C. The concentration of yeast cells was monitored by optical absorbance at 610 nm. The amount of dry biomass in the suspension was determined by samples taken at different intervals from the medium, which were then centrifuged at 4000 rpm for 10 min. and the precipitate was dried at 85°C until it reached the constant weight.

Effect of Ca, Mg and K on the survival of cell in different heavy metal concentrations

The experiment was carried out as mention above, and the rate of the yeast cell growth was measured after the application of $Ca(HCO_3)_2$, $MgSO_4$, or/and K_2SO_4 at 50, 75, and 150 mM, respectively, to all cultures at different concentrations of the investigated heavy metal at 28°C.

The data reported are mean values of three replicates with relative standard deviations. LSD analysis was also carried out, followed by the Tukey's test at 95% in order to assess the significance of the differences and correlation.

Results

Kinetic study of cell growth in different heavy metal

Fig. 1.a and b. show the kinetics of growth of the two *S. cerevisiae* strains (NSS5099 and NSS7002) in the presence of different heavy metals at 50 µM. The highest growth was observed for the yeast strain NSS7002.
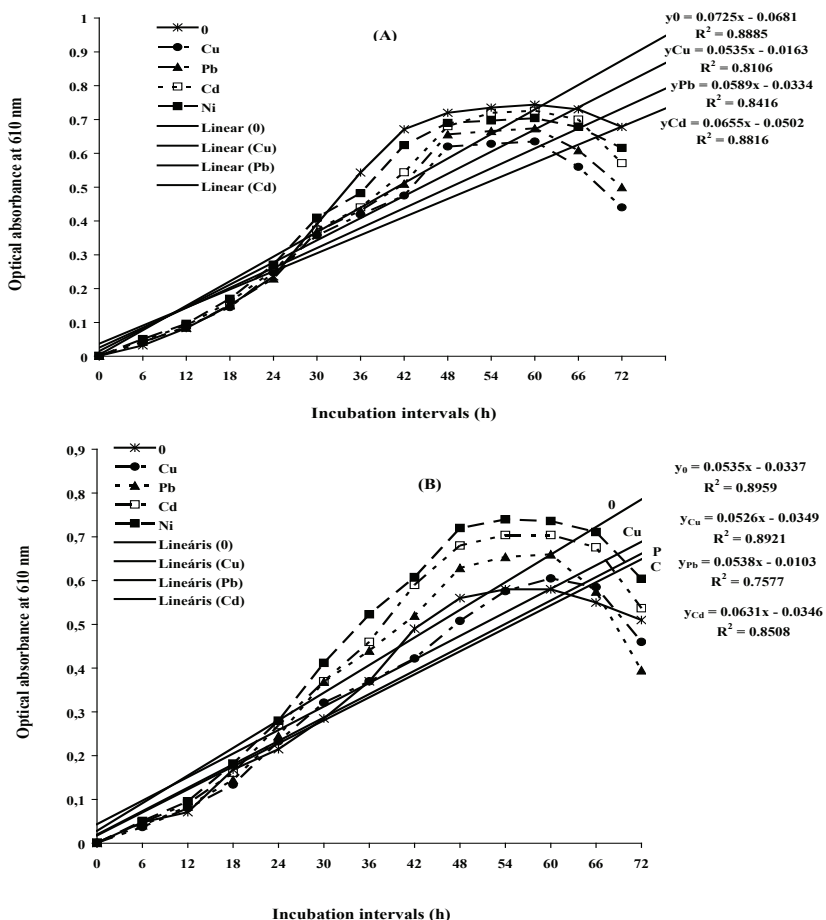

Figure 1. Kinetic growth curve of *Saccharomcyes cerevisiae* strains NSS7002 (A) and NSS0599 (B) in YPD broth medium containing 50 µM of heavy metal after incubation at 150 rpm and 28°C for 48 h.
1. ábra Az NSS7002 (A) és az NSS0599 (B) *S. cerevisiae* törzsek szaporodási kinetikája a nehézfémet 50 µM koncentrációban tartalmazó YPD tápközegben, 150 rpm-en, 28°C-on, 48 órán keresztül történő inkubálást követően

The growth of biomass in the initial period was higher for NSS7002 than NSS5099, but after 24 h it was found a little slower than NSS5099. At the same time, the effect of Cu was more toxic than Pb, Cd and Ni. The stationary phase was developed for NSS5099 strain faster than for NSS7002 strain between 48 and 66 h, and it was depended on the metal ion type.

Determination of cell growth in different heavy metal concentrations
Cu^{2+}, Cd^{2+}, Pb^{2+} and Ni^{2+} ions were examined in connection with inhibition of the cell growth of the two *S. cerevisiae* strains at concentrations from 50 to 500 µM, which showed that at concentrations higher than 300 and 400 µM of Cu^{2+}, Cd^{2+}, Pb^{2+} and Ni^{2+}, respectively, the cell growth significantly decreased.
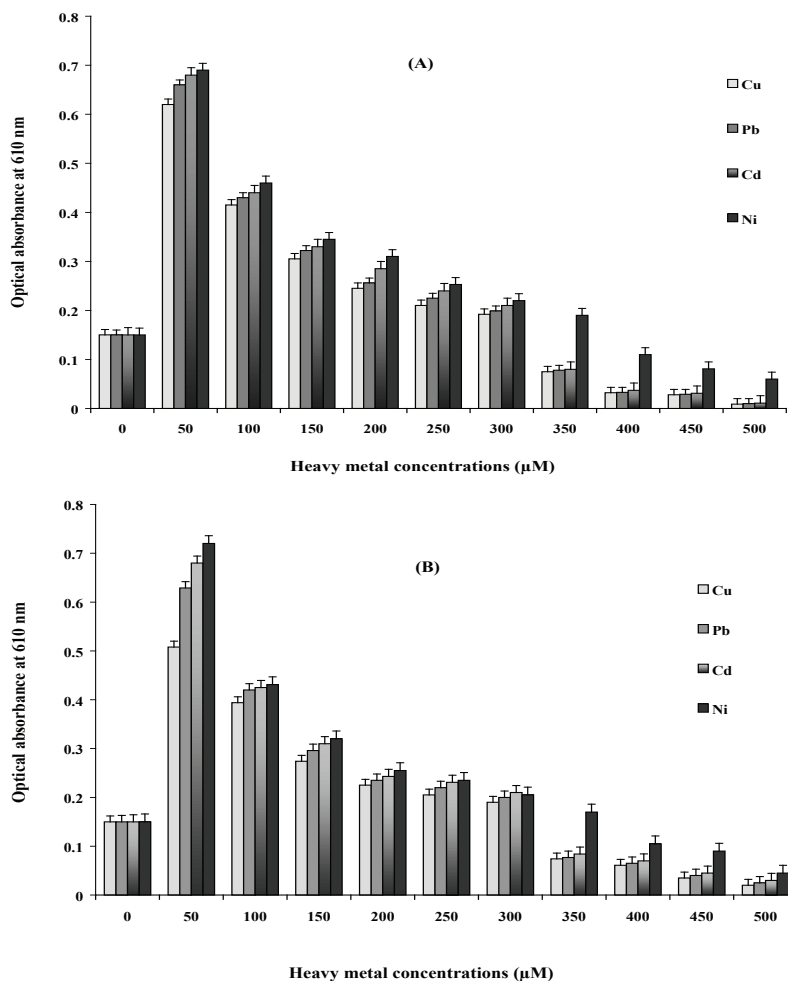

Figure 2. Toxic effects of Cu, Pb, Cd and Ni on the growth of *Saccharomyces cerevisiae* strains NSS7002 (A) and NSS0599 (B) in YPD broth medium after incubation at 150 rpm and 28°C for 48 h.
2. ábra A Cu, Pb, Cd és Ni NSS7002 (A) és NSS0599 (B) *Saccharomyces cerevisiae* törzsekre gyakorolt toxicitása YPD tápközegben, 150 rpm-en, 28°C-on, 48 órán keresztül történő inkubálást követően

Figure 2.a and b. illustrate that the beginning of stronger inhibiting effect of at 350 for Cu^{2+}, Cd^{2+} and Pb^{2+}, but for Ni^{2+} it was at 450 µM. At 150 µM of Cu^{2+}, Cd^{2+}, Pb^{2+} and Ni^{2+}, the effect was found to be negligible. The toxic effect abilities of the heavy metals to the two strains of yeasts were compared. In addition, it was found that the growth curves in case of Cu and Pb were very closed, especially at high concentrations. The results showed that the heavy metals studied could be arranged in the following order according to their toxic effect on both yeast strains: $Cu^{2+} > Pb^{2+} > Cd^{2+} > Ni^{2+}$.

Effect of Ca^{2+}, Mg^{2+} and K^+ on the survival of cell in different heavy metal levels

The results of this experiment showed that the correction of the heavy metal toxicity occurred by the addition of 50 mM $Ca(HCO_3)_2$ in the medium before addition of 350 µM Cu^{2+}, Pb^{2+}, Cd^{2+} or 450 µM Ni^{2+} which show a protective action against cell death and decreased the toxicity effect. The addition of 75 mM $MgSO_4$ and the addition of 150 mM K_2SO_4 reduced the toxic effect of all investigated metals by 40% (Fig. 3).
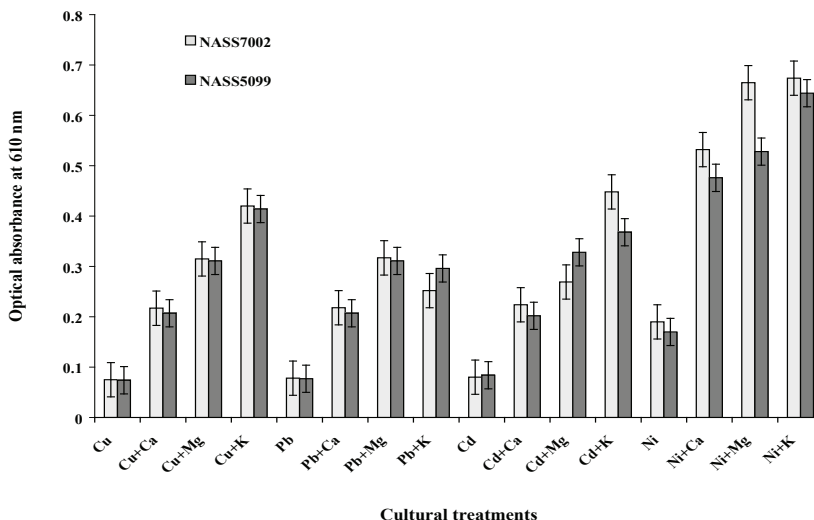

Figure 3. Effect of Ca (50 mM), Mg (75 mM) and K (150 mM) salts on the heavy metal tolerance of *Saccharomyces cerevisiae* strains growing in YPD broth medium containing 350 µM of Cu, Pb or Cd or 450µM of Ni after incubation at 150 rpm and 28°C for 48 h.
3. ábra A Ca- (50 mM), Mg- (75 mM) és K- (150 mM) sók hatása a *S. cerevisiae* törzsek nehézfémekkel szembeni toleranciájára, Cu-, Pb- és Cd- (350 µM), valamint Ni- (450 µM) ionokat tartalmazó tápközegben, 150 rpm-en, 28°C-on, 48 órán keresztül történő inkubálást követően

Effect of Ca^{2+}, Mg^{2+} and K^+ on cell biomass grown at toxic concentrations of heavy metal

Figure 4. shows the mode of correction according to the application of Ca^{2+}, Mg^{2+} and K^+ to growth medium in the combination with the toxic dose of the heavy metal. The results illustrated that the yeast cell dry weight increased by the presence of the alkaline metal as correctors. At the same time, it was increased between 4.5 and 6 times of the cells biomass which grown in polluted medium.
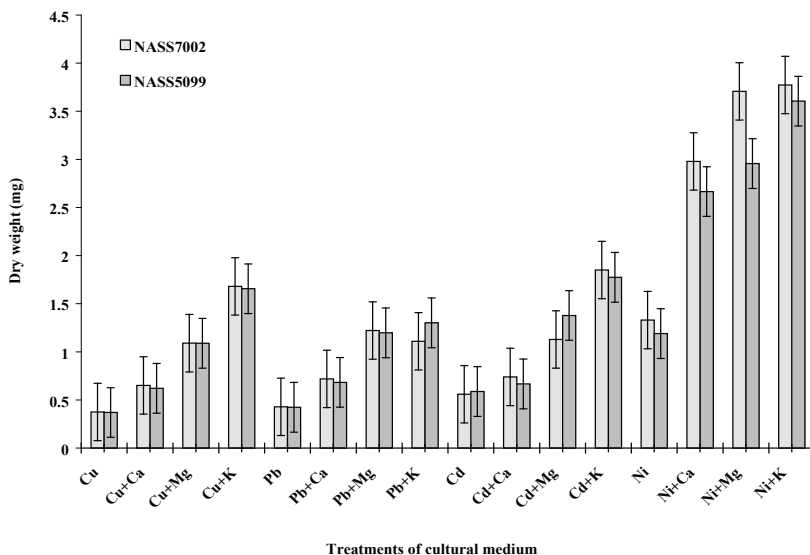
Figure 4. Effect of Ca (50 mM), Mg (75 mM) and K (150 mM) salts on the dry mass (mg) of *Saccharomyces cerevisiae* strains growing in YPD broth amended with toxic doses (350 μM) of Cu, Pb or Cd or 450 μM of Ni after incubation at 28°C for 48 h.

4. ábra A Ca- (50 mM), Mg- (75 mM) és K- (150 mM) sók hatása a Cu-, Pb- és Cd- (350 μM), valamint Ni- (450 μM) iont tartalmazó YPD tápközegben szaporodó *S. cerevisiae* törzsek száraztömegére (mg), 28°C-on, 48 órán keresztül történő inkubálást követően

According to our results and due to the resistance of the two yeast strains it can be mentioned that yeast resistant strain can act as heavy metal bioremediator, the yeast cells can act essentially in two ways: binding the cations to the cell surface (biosorption) and accumulation of cations inside the cell via metabolic transport.

Discussion

Heavy metal pollution has become one of the most serious environmental problems today. Biosorption (using biomaterials e.g., bacteria, fungi, yeast and algae) is regarded as a cost-effective biotechnology for the treatment of high volume and low concentration complex wastewaters containing heavy metal(s) in the order of 1 to 100 mg l^{-1}. Among the promising biosorbents for heavy metal removal which have been researched during the past decades, *S. cerevisiae* has received increasing attention due to the unique nature in spite of its mediocre capacity for metal uptake compared with other fungi (Wang and Chen 2006).

The goal of this investigation was to study the effects of Ca^{2+} and Mg^{2+} on reducing the heavy metals toxicity on yeast grew in cultural medium contaminated by different concentrations of Cu^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} in comparison with the activity of K^+ ions. *S. cerevisiae* as a promising biosorbent has been used to remove Cr^{6+} and Fe^{3+} (Goyal et al. 2003), Cd^{2+} (Liu et al. 1997), Cu^{2+} (Machado et al. 2009) from aqueous solutions. Moreover, it can distinguish different metal species based on their toxicity, such as selenium

(Se), antimony (Sb) and Hg. Gad et al. (2010) mentioned that the working concentrations used in the biosorption medium of *S. cerevisiae* was 35 mgl^{-1}, Cu^{2+}; 15 mgl^{-1},Cd^{2+} and 25 mgl^{-1}, Fe^{3+}. 18h equilibrium time needed for maximum metal removal, (100 mgl^{-1}) metals adapted S. *cerevisiae* hasn't prominent enhancing effect, while addition of (4 mgl^{-1}) cystine has such effect upon metal removal, with 5.5 initial pH, and 3% (v/v) inoculums concentration while NaOH treatment resulted in 36.11, 18.11, 33.52% for Cu^{2+},Cd^{2+} and Fe^{3+}, respectively.

Microorganisms have had to develop different mechanism of metal resistance that include cell membrane metal efflux, intracellular chelation by metallothionine protein and glutathione derived peptides called phytochelatins and metal artmentalization in vacuoles (EL AASAR 2005). Other mechanisms exist for the removal of heavy metals from aqueous solution by bacteria, fungi, ciliates, algae, mosses, macrophytes and higher plants (REHMAN et al. 2008). As cellular response to the presence of metals such as biosorption by cell biomass, active cell transport, binding by cytosolic molecules, entrapment into cellular capsule, precipitation and oxidation-reduction reactions (LOVELY and COATES, 1997). Metal uptake is dependent not only on the type or species of microorganisms, but on growth conditions influence considerably the composition of yeast cells and thereby the binding abilities of cells for metal ions (DOSTALEK et al. 2004). RUTA et al. (2010) studied the heavy metal hypersensitive yeast strain *pmr1Δ* for the ability to remove Mn^{2+}, Cu^{2+}, Co^{2+}, or Cd^{2+} from synthetic effluents. Due to increased metal accumulation, the mutant strain was more efficient than the wild-type in removing Mn^{2+}, Cu^{2+}, or Co^{2+} from synthetic effluents containing 1–2 mM cations, with a selectivity Mn^{2+} > Co^{2+} > Cu^{2+} and also in removing Mn^{2+} and Cd^{2+} from synthetic effluents containing 20–50 μM cations, with a selectivity Mn^{2+} > Cd^{2+}.

The phenomenon of biosorption is defined as a metabolism independent adsorption of pollutants based on the partition process on a microbial biomass (RINGOT et al. 2007). Or, it is a passive non-metabolically-mediated process of metal binding by biosorbent (DAVIS et al. 2003). GNIEWOSZ et al. (2007) investigated the Mg^{2+} biosorption by waste brewery *S. uvarum*. According to our results, this could one of the reasons for cell protection. CHEN and WANG (2007) found that Pb at 2 μM did not affect the growth of *S. cerevisiae*, but 5 μM only inhibited 30% and 10 μM of Pb inhibited 50% of total growth of *S. cerevisiae*. The authors gave a toxicity order of the tested metals on the growth of *S. cerevisiae* as Pb^{2+} > Ag^{+} > Cr^{3+} > Cu^{2+} > Zn^{2+} > Cd^{2+} > Co^{2+} > Sr^{2+} > Ni^{2+} > Cs^{+}. But our strains had the ability to tolerate higher doses (Figs. 1-3) than those mentioned by the authors. PASTERNAKIEWICZ (2006) found that more than 50 μM Cd^{2+} concentrations had negative effect on yeast growth but, this effect was corrected by the presence of Ca^{2+}. Our findings supported the conclusion of PASTERNAKIEWICZ (2006). BLACKWELL et al. (1998) mentioned that Mg^{2+}, Ca^{2+} and K^{+} reduced the Mn^{2+} toxicity towards *S. cerevisiae*. The toxic effects of investigated metals here, to some extent were similar to our observations in the present study (Figs. 4−5). Concomitant with metal uptake, ion release from biomass (viable and inactivated) is frequently observed. Release of K^{+}, H^{+}, Ca^{2+} and Mg^{2+} (AVERY and TOBIN 1993; BRADY & DUNCAN 1994) has been most studied. There are varying reports on whether a stoichiometric relationship exists between ion release and metal uptake. Numerous studies refer to K^{+} release by yeast in response to metal uptake. Ca^{2+} accumulation by *S. cerevisiae* resulted in rapid release of 70% of cellular K^{+}, followed by a slower release of approximately 60% of cellular Mg^{2+}, but little loss of Ca^{2+} (BRADY and DUNCAN

1994). Gadd and Mowll (1983) also reported the absence of a simple stoichiometric relationship between Cd^{2+} uptake and K^+ release. K^+ release was attributed to membrane disruption by Cd^{2+} binding to organic ligands, and was more marked in the presence of glucose. Cu^{2+} and Cd^{2+} accumulation induced extensive loss of cellular K^+ and Mg^{2+} but little loss of Ca^{2+} (Brady and Duncan 1994). Li^+ accumulation was accompanied by a stoichiometric efflux of K^+ (Perkins and Gadd 1993). Also two K^+ ions were released for each Cd^{2+} ion accumulated intracellularly (Mowll and Gadd 1984) suggesting K^+ efflux occurs to maintain ionic balance across the membrane.

The potential of yeasts for accumulating a range of metal cations from aqueous solutions is well known. Therefore, heavy metals inhibitory effects on *S. cerevisiae* were to be studied. Low concentrations of certain heavy metal ions are necessary for the vitality of all microbial cells. Low concentrations of Cu^{2+} and Zn^{2+} even stimulate the growth and the activity of the metabolic process. At high heavy metal concentration, the growth may be restrained. Non-essential metals such as Cd^{2+} and Pb^{2+} can interact with fungal cells and be accumulated by physico-chemical mechanisms. This non-essential metal exhibits toxicity above certain concentrations, which varies within the fungal species, the physico-chemical properties of the metal and environmental factors (Gadd 1992). Our results demonstrated that the metal exhibit toxicity above certain concentrations, which varies within the levels of the yeast microbial strains. Wang and Chen (2006) extensively discussed the characteristics of *S. cerevisiae* in heavy metal biosorption field and summarized various mechanism assumptions of metal uptake by *S. cerevisiae*. Authors mentioned that yeast biosorption largely depends on parameters such as pH, the ratio of the initial metal ion and initial biomass concentration, culture conditions, presence of various ligands and competitive metal ions in solution and to a limited extent on temperature. Soares et al. (2003) stated that the decreasing order of toxicity of select heavy metals on the *S. cerevisiae*, in 10 mM MES (2-(*N*-morpholino)-ethane-sulfonic acid) pH buffer at pH 6.0, was found to be Cu^{2+}, Pb^{2+}, and Ni^{2+}. Heavy metal (200 µM) induced a decrease in the number of viable cells by about 50% in the first 5 min for Cu^{2+} and in 4 h for Pb^{2+}, while Ni^{2+} was not toxic up to a 200 µM concentration over a period of 48 h. The addition of 0.5 mM Ca^{2+}, before addition of 200 µM Cu^{2+}, showed a protective action against cell death, while no effect was observed against Pb^{2+} or Ni^{2+} toxic effects. The present study is confirmed by these conclusions. Nakamura et al. (2007) investigated the influence on the colorimetric response of dissolved inorganic ions (Cu^{2+}, Mn^{2+}, Zn^{2+}, Cr^{3+} and Fe^{3+}) in natural water. Karamushka et al. (1998) indicated that the toxicity of heavy metal ions correlated with their inhibiting effect on transmembrane potential or parameters related to the operation of the generators of transmembrane potential of microbial cells. Blackwell et al. (1997) concluded that Mn^{2+} uptake and toxicity in *S. cerevisiae* are strongly influenced by intracellular Mg^{2+}, possibly through Mg-dependent regulation of divalent cation transport activity. Our results are in agreement with this investigational comment. A commercial strain of *S. cerevisiae* was serially cultured in media containing Cu^{2+} up to a final concentration of 10 mmol l^{-1}. This Cu-tolerant subculture was assessed for its capacity to accumulate further quantities of Cu^{2+}. It was found that after Cu^{2+} accumulation the total Cu content of this yeast was lower than the parent culture when exposed to similar conditions, indicating that the subculture was Cu-resistant owing to reduced Cu bioaccumulation properties (Brady et al. 1994).

Viability of biomass during the course of experiments is important when considering metabolism-dependent metal uptake (GADD and MOWLL 1983). WHITE and GADD (1987) observed a progressive reduction in viability (to 50%) of cells incubated over a range of Zn^{2+} concentrations up to 100 ~M. Viability loss correlated with indicators of Zn^{2+} toxicity such as inhibition of H^+ efflux and K^+ uptake but not with Zn^{2+} uptake. K^+ efflux was uniphasic with little K^+ efflux observed in the presence of factors that decreased Cu^{2+} uptake into cells. Levels of intracellular K^+ were critical for maximal Cu^{2+} accumulation-cells with high levels of internal K^+ (approx. 139 mM) took up more Cu^{2+} than cells with lower levels. Greater amounts of Mg^{2+} were released by denatured yeast (5 to 40 fold greater) but Ca^{2+} and H^+ displacement was reduced. Cytoplasmic levels of Ca^{2+} and Mg^{2+} declined in response to Sr^{2+} uptake. Further Mg^{2+}, but not Ca^{2+}, loss from the vacuole correlated with stimulated Sr^{2+} uptake in the presence of glucose.

Survival of microorganisms in the presence of toxic metals depends on intrinsic biochemical and structural properties, physiological and/or genetic adaptation, environmental modification of metal speciation, availability and toxicity. Most of the bioremediation studies involving *S. cerevisiae* focused mainly on cell surface properties, i.e., biosorption by dead biomass (MACHADO et al. 2008, 2009) by living cells with improved biosorption capacity (SALEEM et al. 2008) or with engineered cell surface (SHIBASAKI et al. 2009; KURODA et al. 2001; KAMBE-HONJOH et al. 2000).

K^+ and Mg^{2+} were found to inhibit Zn^{2+} uptake by *S. cerevisiae* whereas Na^+ and Ca^{2+} did not. It was concluded that inhibition of Zn^{2+} uptake was related to K^+ and Mg^{2+} accumulation by the cell (Ca^{2+}, Na^+ were not taken up). Reduction of net surface charge by bound cation may also be involved (BORST-PAUWELS and THEUVENET 1984). In contrast, the presence of Ni^{2+} enhanced Zn^{2+} uptake at concentrations of 100 μM compared to 20 μM Zn but its mode of action was unknown (WHITE and GADD 1987). Ca^{2+} uptake by yeast was inhibited by monovalent cations (K^+, Rb^+, Cs^+, Na^+ and Li^+) at equimolar concentrations, and correlated with uptake of these cations (ROOMANS et al. 1979). Ca^{2+} was found to depress Cd^{2+} uptake strongly. MOWLL and GADD (1984) reported similar findings and proposed that Cd^{2+} was accumulated via a Ca^{2+} transport system.

In conclusion, strains of *S. cerevisiae* have contributed to our understanding of metal uptake and toxicity. In a biotechnological context, yeasts may be useful in the treatment of metal-containing effluents. Finally, the presence of 350 μM of Cu^{2+}, Pb^{2+}, Cd^{2+} and 450 μM Ni^{2+} in growth medium had a negative effect on the growth of both yeast strains. The negative effect of higher concentrations of Cu^{2+}, Pb^{2+}, Cd^{2+} and Ni^{2+} was lowered by the addition of Mg^{2+}, Ca^{2+} and K^+. The results obtained in the present study exhibit the higher potentiality of *S. cerevisiae* strain NSS7002 than the strain NSS5099 to be used for decontamination of soil containing heavy metal ions. Further task is going to examine the range of metal bioaccumulation in the yeast cells and the ability of these strains to be environmental bioremediators.

References

Akhtar N., Iqbal J., Iqbal M. 2004: Removal and recovery of nickel(II) from aqueous solution by loofa sponge-immobilized biomass of *Chlorella sorokiniana*: characterization studies. J Hazard Mater. 108: 85−94.

Avery S.V., Tobin J.M. 1993: Mechanisms of adsorption of hard and soft metal ions to *Saccharomyces cerevisiae* and influence of hard and soft anions. Appl. Environ. Microbiol., 59: 2851−2856.

Avery S.V., Howlett N.G., Radice S. 1996: Copper toxicity towards *Saccharomyces cerevisiae*: dependence on plasma membrane fatty acid composition. Appl. Environ. Microbiol. 62: 3960−3966.

Bender J., Phillips P. 2004: Microbial mats for multiple applications in aquaculture and bioremediation. Biores. Technol. 94: 229−238.

Borst-Pauwels G., Theuvenet A. 1984: Apparent saturation kinetics of divalent cation uptake in yeast caused by a reduction in the surface potential. Biochem. Biophys. Acta 771: 171−176.

Blackwell K.J., Tobin J.M., Avery S.V. 1997: Manganese uptake and toxicity in magnesium-supplemented and unsupplemented *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 47: 180−184.

Blackwell K.J., Tobin J.M., Avery S.V. 1998: Manganese toxicity towards *Saccharomyces cerevisiae*: Dependence on intracellular and extracellular magnesium concentrations. Appl. Microbiol. Biotechnol. 49: 751−757.

Brady D., Duncan J.R. 1994: Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 34: 149−154.

Brady D., Glaum D., Duncan J.R. 1994: Copper tolerance in *Saccharomyces cerevisiae*. Lett. Appl. Microbiol. 18: 245−250.

Can C., Jianlong W. 2007: Correlating metal ion characteristics with biosorption capacity using QSAR model. Chemosphere 69: 1610−1616.

Chen C.M, Wang J. 2007: Response of *Saccharomyces cerevisiae* to lead ion stress. Appl. Microbiol. Biotechnol. 74: 683−687.

Chojnacka K. 2010: Biosorption and bioaccumulation–the prospects for practical applications. Environ. International 36: 299−307.

Collins Y.E., Stotzky G. (1992): Heavy metals alter the electrokinetic properties of bacteria, yeasts, and clay minerals. Appl. Environ. Microbiol. 58: 1592−1600.

Davis T.A., Volesky B., Mucci A. 2003: A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 37: 4311−4330.

Dostalek P., Patzak M., Matejka P. 2004: Influence of specific growth limitation on biosorption of heavy metals by *Saccharomyces cerevisiae*. Intern. Biodeterior. Biodegr. 54: 203−207.

El Aasar S.A. 2005: Adaptive tolerance of *Trichoderma hamatum* in cadmium, copper and lead heavy metals. Egypt J. Biotechnol. 21: 278−294.

Engl A., Kunz B. 1995: Biosorption of heavy metals by *Saccharomyces cerevisiae*: effects of nutrient conditions. J. Chem. Technol. Biotechnol. 63: 257−261.

Gad S.A., Attia M., Ahmed A.H. 2010: Heavy Metals Bio-Remediation by Immobilized *Saccharomyces cervisiae* and *Opuntia ficus indica* Waste J. Am. Sci. 6: 79−87.

Gadd G.M. 1992: Metals and microorganisms: a problem of definition. FEMS Microbiol. Lett. 100: 197−204.

Gadd G.M. 1993: Interactions of fungi with toxic metals. New Phytol. 124: 25−60.

Gadd G.M., Mowll J.L. 1983: The relationship between cadmium uptake, potassium release and viability in *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. 16: 45−48.

Giller K.E., Witter E., McGrath S.P. 1997: Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils. Soil Biol. Biochem. 29: 1389−1414.

Gniewosz M., Duszkiewicz-Reinhard W., Blazejak S., Sobiecka J., Zarzecka M. 2007: Investigations into magnesium biosorption by waste brewery yeast *Saccharomyces uvarum*. Acta Sci. Pol., Technol. Aliment. 6: 57−67.

Goyal N., Jain S.C., Banerjee U.C. 2003: Comparative studies on the microbial adsorption of heavy metals. Adv. Environ. Res. 7: 311−319.

Hiroki M. 1992: Effects of heavy metal contamination on soil microbial populations. Soil Sci. Plant Nutr. 38: 141−147.

Horvath J. 1970: Microbiology. Mezőgazdasági Kiadó, Budapest.

Huang C.P., Morehart A. 1990: The removal of Cu(II) from dilute aqueous solutions by *Saccharomyces cerevisiae*. Water Res. 4: 433−439.

Jianlong W. 2002: Biosorption of copper (II) by chemically modified biomass of *Saccharomyces cerevisiae*. Process Biochem. 37: 847−380.

KAMBE-HONJOH H., OHSUMI K., SHIMOI H., NAKAJIMA H., KITAMOTO K. 2000: Molecular breeding of yeast with higher metal-adsorption capacity by expression of histidine-repeat insertion in the protein anchored to the cell wall. J. Gen. Appl. Microbiol. 46: 113−117.

KAPOOR A., VIRARAGHAVAN T. 1997: Heavy metal biosorption sites in *Aspergillus niger*. Biores. Technol. 61: 221−227.

KURODA K., SHIBASAKI S., UEDA M., TANAKA A. 2001: Cell surface engineered yeast displaying a histidine oligopeptide (exa-His) has enhanced adsorption of and tolerance to heavy metal ions. Appl. Microbiol. Biotechnol. 57: 697−701.

KARAMUSHKA V.I., GADD D.M., GRUZINA T.G., UL'BERG Z.R. 1998: The application of colloid-biochemical parameters of microbial cells for the assessment of heavy metal toxicity. Colloid J. Russ. Acad. Sci. 60: 717−720.

LIU X.F., SUPEK F., NELSONI N., CULOTTA V.C. 1997: Negative control of heavy metal uptake by the *Saccharomyces cerevisiae* BSD2 Gene. J. Biol. Chem. 272: 11763−11769.

LLOYD J.R., LOVLEY D.R., MACASKIE L.E. 2003: Biotechnological application of metal-reducing microorganisms. Adv. Appl. Microbiol. 53: 85−128.

LO W., CHUA H., LAM K.H. 1999: A comparative investigation on the biosorption of lead by filamentous fungal biomass. Chemosphere 39: 2723−2736.

LOVELY D.R., COATES J.D. 1997: Bioremediation of metal contamination. Curr. Opin. Biotechnol. 8: 285−289.

MACHADO M.D., JANSSENS S., SOARES H.M.V.M., SOARES E.V. 2009: Removal of heavy metals using a brewer's yeast strain of *S. cerevisiae*: advantages of using dead biomass. J. Appl. Microbiol. 106: 1792−1804.

MACHADO M.D., SANTOS M.S., GOUVEIA C., SOARES H.M., SOARES E.V. 2008: Removal of heavy metals using a brewar's yeast strain of *Saccharomyces cerevisiae*: the flocculation as a separation process. Biores. Technol. 99: 2107−2115.

MALIK A. 2004: Metal bioremediation through growing cells. Environ. Int. 30: 261−278.

MOWLL J.L., GADD G.M. 1984: Cadmium uptake by *Aureobasidium pullulans*. J. Gen. Microbiol. 130: 279−284.

NAEEM A., WOERTZ J.R., FEIN J.B. 2006: Experimental measurement of proton, Cd, Pb, Sr and Zn adsorption onto the fungal species *Saccharomyces cerevisiae*. Environ. Sci. Technol. 40: 5724−5729.

NAKAMURA H., HIRATA Y., MOGI Y., KOBAYASHI S., SUZUKI K., HIRAYAMA T., KARUBE I. 2007: A simple and highly repeatable colorimetric toxicity assay method using 2,6-dichlorophenolindo-phenol as the redox color indicator and whole eukaryote cells. Anal. Bioanal. Chem. 389: 835−840.

PARK J.K., LEE J.W., JUNG J.Y. 2003: Cadmium uptake capacity of two strains of *Saccharomyces cerevisiae* cells. Enzyme Microbiol. Technol. 33: 371−378.

PASTERNAKIEWICZ A. 2006: The growth of *Saccharomyces cerevisiae* yeast in cadmium enriched media. Acta Sci. Pol. Technol. Aliment. 5: 39−46.

PERKINS J., GADD G.M. 1993: Accumulation and intracellular compartmentation of lithium ions in *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. 107: 255−260.

REHMAN A., SHAKOORI F.R., SHAKOORI A.R. 2008: Heavy metal resistant freshwater ciliate, Euplotes mutabilis, isolated from industrial effluents has potential to decontaminate wastewater of toxic metals. Biores. Technol. 99: 3890−3895.

RINGOT D., LERZY B., CHAPLAIN K., BONHOURE J.P., AUCLAIR E., LARONDELE Y. 2007: *In vitro* biosorption of ochratoxin A on the yeast industry byproducts: Comparison of isotherm models. Biores. Technol. 98: 1812−1821.

ROOMANS G., THEUVENET A., VAN DEN BERG T., BORST-PAUWELS G. 1979: Kinetics of Ca^{2+} and Sr^{2+} uptake by yeast. Effects of pH, cations and phosphate. Biochim. Biophys. Acta 551: 187−196.

RUTA L., PARASCHIVESCU C., MATACHE M., AVRAMESCU S., FARCASANU I.C. 2010: Removing heavy metals from synthetic effluents using "kamikaze" Saccharomyces cerevisiae cells. Appl. Microbiol. Biotechnol. 85:763−771.

SALEEM M., BRIM H., HUSSAIN S., ARSHAD M., LEIGH M.B., ZIA-UL H. 2008: Perspectives on microbial cell surface display in bioremediation. Biotechnol. Adv. 26: 151−161.

SALTUKOGLU A., SLAUGHTER J.C. 1983: The effect of magnesium and calcium on yeast growth. J. Inst. Brew. 89: 81−83.

SHIBASAKI S., MAEDA H., UEDA M. 2009: Molecular display technology using yeast-arming technology. Anal. Sci. 25: 41−49.

SINGLETON I., SIMMONS P. 1996: Factors affecting silver biosorption by and industrial strain of *Saccharomyces cerevisiae*. J. Chem. Tech. Biotechnol. 65: 21−28.

SOARES E.V., HEBBELINCK K., SOARES H.M.V.M. 2003: Toxic effects caused by heavy metals in the yeast *Saccharomyces cerevisiae*: a comparative study. Can. J. Microbiol. 49: 336−343.

Suh J.H., Kim D.S., Yun J.W., Song S.K. 1998: Process of Pb²⁺ accumulation in *Saccharomyces cerevisiae*. Biotecnol. Lett. 20: 153−156.

Tuszynski T., Pasternakiewicz A. 2000: Bioaccumulation of metal ions by yeast cell of *Saccharomyces cerevisiae*. Pol. J. Food Nutr. Sci. 4: 31−39.

Veglio F., Beolchini F. 1997: Removal of metals by biosorption. Hydrometallurgy 44: 301−316.

Volesky B., Phillips H.A. 1995: Biosorption of heavy metals by *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 42: 797−806.

Wang J., Chen C. 2006: Biosorption of heavy metals by *Saccharomyces cerevisiae*. Biotechnol. Adv. 24: 427−451.

White C., Gadd G.M. 1987: The uptake and cellular distribution of zinc in *Saccharomyces cerevisiae*. J. Gen. Microbiol. 133: 727−737.

Wang J., Chen C. 2009: Biosorbents for heavy metals removal and their future. Biotechnol. Adv. 27: 195−226.

Zouboulis A. I., Matis K. A., Lazaridis N.K. 2001: Removal of metal ions from simulated wastewater by *Saccharomyces* yeast biomass: combining biosorption and flotation processes. Separation Sci. Technol., 36: 349−365.

KÉTÉRTÉKŰ ALKÁLIFÉMEK HATÁSA AZ ÉLESZTŐSEJTEK SZAPORODÁSÁRA, NEHÉZFÉMEKKEL SZENNYEZETT TALAJBAN

[1]BAYOUMI HAMUDA HOSAM E.A.F. [2]TÓTH NIKOLETT

[1]Óbudai Egyetem, Rejtő Sándor Könnyűipari és Környezetmérnöki Kar, Környezetmérnöki Intézet
1034 Budapest, Doberdó út 6., e-mail: hosameaf@gmail.com.
[2]Szent István Egyetem, Környezettudományi Doktori Iskola. 2100 Gödöllő, Páter K. u. 1.

Kulcsszavak: alkáli- és nehézfémek, *Saccharomyces cerevisiae,* szaporodás, toxicitás, talajszennyezés

Összegzés: A mikroorganizmusok tulajdonságai nagyon jól hasznosíthatóak a talajszennyezés monitorozásánál. A toxikus nehézfémek komoly ökológiai problémát jelentenek környezetünkben, ezért kiemelkedő fontosságú a nehézfémekkel szennyezett talajok tisztítása. *In vitro,* két *S. cerevisiae* törzs (NSS5099 és NSS7002) nehézfémekkel szembeni toleranciáját vizsgáltuk. A két törzs szaporodási kinetikáját olyan táptalajon tanulmányoztuk, amelyhez 50 µM koncentrációban adtunk Cu²⁺-, Pb²⁺-, Cd²⁺- vagy Ni²⁺-ionokat. A vizsgált nehézfémek élesztőtörzsekre gyakorolt toxicitása csökkenő sorrendben: Cu²⁺ > Pb²⁺ > Cd²⁺ > Ni²⁺. A 350 µM koncentrációjú Cu²⁺, Pb²⁺ vagy Cd²⁺ és 450 µM koncentrációjú Ni²⁺ 48 órás inkubációt követően 50%-kal csökkentette az élősejtek számát. Amikor a nehézfémek táptalajba történő adagolása előtt 50 mM Ca(HCO₃)₂, 75 mM MgSO₄, vagy 150 mM K₂SO₄-ot adtunk a közeghez csökkent a nehézfémek sejtekre gyakorolt toxicitása, és több sejt maradt életben. A 350 és 450 µM koncentrációban lévő nehézfémek toxicitását a fémsók 40%-kal csökkentették. A kapott eredmények alapján az NSS7002 törzs sokkal alkalmasabbnak bizonyult a nehézfémekkel szennyezett talajok tisztítására, mint az NSS5099.