

## INVESTIGATIONS INTO MAGNESIUM BIOSORPTION BY WASTE BREWERY YEAST *SACCHAROMYCES UVARUM*

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**Abstract.** Investigations were carried out into the capacity of waste brewery yeast *Saccharomyces uvarum* for biosorption of magnesium originated from a solution of dehydrated salt of magnesium chloride, depending on the number of cells and different pH of the suspension during 6 hours. The concentration of  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  in the solution was adjusted so as to maintain a stable content of magnesium as expressed per pure element, i.e.  $1.25 \text{ g/dm}^3$  of solution. In the first stage, the number of cells was differentiated in yeast slurry through either condensation or dilution. In the second stage, pH of yeast suspension was differentiated (pH 5.5, 6.0 and 7.0) at a constant number of cells. The solutions examined were kept under anaerobic and aerobic conditions. Determination of magnesium content of yeast biomass was carried out with the method of atomic adsorption spectroscopy after 15 min, 1 h, 2 h, 4 h and 6 h of experiments. The highest content of magnesium (13.76 mg/g d.m.) was obtained at the lowest number of cells in the solution, i.e.  $3.5 \times 10^8$  cells/cm<sup>3</sup> under aerobic conditions. An increase in solution pH facilitated biosorption of magnesium by the yeast. At pH 7.0, after 6 hours of the experiment, the yeasts contained 15.19 mg Mg/g d.m. when kept under anaerobic conditions and 17.22 mg Mg/g d.m. when kept under aerobic conditions.

**Key words:** magnesium, brewery yeast, *Saccharomyces uvarum*

### INTRODUCTION

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  $\text{Mg}^{2+}$  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 cell wall constitutes from 15 to 30% of d.m. of yeast biomass and from 25 to 50% of cell volume, and its thickness ranges from 10 to 70 nm [Brady et al. 1994, Orlean 1997]. It is built mainly of  $\beta$ -1,3-glucan (up to 50% of wall

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d.m.) and mannoproteins (up to 40%).  $\beta$ -1,6-Glucan and chitin occur in lesser amounts (up to 10% and 1%, respectively) [Lipke and Ovalle 1998, Kapteyn et al. 2000, Klis et al. 2002]. Mannoproteins constitute the internal layer of the cell wall. They are formed after linking mannan molecules to aspartic residues of proteins by N-acetylglucosamine dimmers [Lipke and Ovalle 1998, Park et al. 2003]. Mannoproteins are highly glycosylated polypeptides (saccharides constitute from 50 to 90% of their molecular weight), hence they are often defined as proteoglycans [Vaart et al. 1995]. Particular modules of mannanoproteins bind between each other, thus additionally strengthening the entire structure of the cell wall.

Absorption of magnesium by yeast cells from the medium is a two-phase process. In the first phase, magnesium is adsorbed to anionic groups occurring on the surface of the cell wall irrespective of cell metabolism and without the need of energy expenditure [Walker 1994, Gardner 2003]. The second phase, metabolically-independent and proceeding considerably slower than the chemisorption on the surface of cell walls, is bioaccumulation. That phase is usually linked with active transport of magnesium through the wall and cytoplasmic membrane to the cell's interior [Blackwell et al. 1995].

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 magnesium biosorption by cells [Tuszyński and Pasternakiewicz 2000, Park et al. 2003, Göksungur et al. 2005]. According to literature data, the optimal temperature range of the biosorption process of magnesium accounts for 25-35°C whereas that of pH for 4.0-8.0 [Thomas 1980, Fourest and Roux 1992, Brady and Duncan 1994]. A low temperature (below 5°C) exerts an inhibiting effect on the process of binding elements by yeast, whereas the capacity of metals biosorption at a temperature range of 5-25°C remains at a similar level [White and Gadd 1987]. As reported by Tuszyński and Pasternakiewicz [1999], pH of the culture medium determines, to a great extent, the competence between hydrogen cations and metal cations for adsorption sites on the external surface of structures of yeast cell wall. The capacity of binding metal ions can also be inhibited by an excessive number of yeast cells present in the medium [Park et al. 2003].

Magnesium absorption by yeast cells may also progress through mechanisms typical of other elements, e.g. the system of  $\text{Fe}^{3+}$  transport. The transport of trivalent iron proceeds with specific ligands – iron-absorptive components. They are assumed to possess affinity also to magnesium and be used by the cell for bioaccumulation of that element. That process ensues slowly and for absorption of  $\text{Mg}^{2+}$  ions it may only be used in the case of their high deficiency [Walker 1994, Emelyanova 2001].

Investigations carried out recently [MacDiarmid and Gardner 1998, Bui et al. 1999, Graschopf et al. 2001, Gardner 2003] have demonstrated that some protein transporters participate in magnesium absorption from the culture medium by yeast, e.g. Alr1p and Alr2p. They belong to the family of MIT proteins (*Metal Ions Transporter*), being constituents of the cytoplasmic membrane [Knoop et al. 2005]. The Alr proteins (proteins of tolerance to aluminium) demonstrate a similar structural affinity to bacterial transporters CorA (proteins of tolerance to cobalt), identified in *Salmonella typhimurium*, that are engaged in transport of  $\text{Mg}^{2+}$  ions [Liu et al. 2002, Hiromura and Sakurai 2001].

Apart from Alr1p and Alr2p transporters, there are also other protein transporters, of which Mrs2p and Lpe10p are involved in the process of magnesium accumulation by yeast [Graschopf et al. 2001, Gegan et al. 2001, Gardner 2003]. They serve a similar

function as protein transporters of magnesium, MgtA and MgtB, occurring in the periplasmic space of Gram-negative bacteria [Hiromura and Sakurai 2001]. Closely related Mrs2p and Lpe10p are responsible for the transport and appropriate concentration of  $Mg^{2+}$  ions in the internal mitochondrial membrane [Knoop et al. 2005].

The process of intracellular accumulation of magnesium may also progress with the share of low-molecular proteins with a high content of cysteine, the so-called "metallothioneins". They are encoded by structural genes and their expression is induced by an increased concentration of metal ions in the medium [Słaba and Długoński 2002]. These low-molecular proteins often participate in detoxication of cells from metals that are bound by cysteine residues.

The reported study examined the capacity of waste brewery yeasts for magnesium biosorption during their suspension for a few hours in a solution of a well-soluble magnesium salt. That treatment is not expensive and is likely to contribute to a fewfold increase in the content of easily available magnesium in yeast cells. In view of the risk of prionic diseases, especially BSE, magnesium-enriched biomass of yeast cells may constitute an element of a feeding programme of animals as a component improving the nutritive value of fodders. In a human diet, natural bioplexes of magnesium obtained from such yeast biomass, as protein-mineral preparations, may diminish deficiency of that element in the body.

## MATERIAL AND METHODS

### Biological material

In the study, use was made of waste slurry of brewery yeast *Saccharomyces uvarum* originating from the Brewery in Warka. The yeasts were kept at a temperature of 4°C in pure tissues rinsed with deionized water. A source of magnesium was  $MgCl_2 \cdot 6H_2O$  solution prepared on deionized water. Solutions of hydrated magnesium chloride were prepared so as to provide a stable concentration of magnesium reaching 1.25 g/dm<sup>3</sup> when expressed per pure element [Błażejczak et al. 2002, Rees and Stewart 1997].

### Preparation of yeast slurry for analyses

In the first stage of the study, biomass of waste brewery yeast was either condensed or diluted in a solution of hydrated magnesium chloride, as a result of which three solutions of yeast slurry were prepared. In variant I, the yeast slurry was condensed ten times. To this end 1000 cm<sup>3</sup> of the slurry were centrifuged in sterile thimbles at 3500 rpm for 10 minutes (Centrifuge 5804R, Eppendorf, France), then the centrifuged biomass was suspended in 10-times smaller volume of an  $MgCl_2 \cdot 6H_2O$  solution in respect of the supernatant obtained. In variant II, the yeast slurry was condensed two times, i.e. 400 cm<sup>3</sup> of the yeast slurry were centrifuged under conditions as described above, and next the centrifuged biomass was suspended in two times smaller volume of an  $MgCl_2 \cdot 6H_2O$  solution in respect of the supernatant obtained. Variant III involved double dilution of the yeast slurry, 100 cm<sup>3</sup> of which were centrifuged at 3500 rpm for 10 minutes and the cells were suspended in two times higher volume of the  $MgCl_2 \cdot 6H_2O$  solution in respect of the supernatant obtained. So prepared solutions were kept at a tem-

perature of 28°C for six hours under aerobic and anaerobic conditions. In order to aerate yeast cells, flasks with the slurry suspended in the  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  solution were fixed on a reciprocating shaker working at 200 rpm (SM-30 Control, E. Bühler, Germany).

In the second stage of the experiments, pH of the yeast slurry solution was changed at a constant number of cells. Three solutions of yeast slurry double diluted in magnesium chloride salt were prepared (as in the variant III) with pH: 5.5, 6.0 and 7.0. Acidity of the solutions was adjusted with 10% HCl or 10% NaOH.

### Analytical methods

The number of yeast cells in the slurry was determined with the plate method, using the YPD culture medium with the following composition (g/L): glucose – 20, peptone – 10, yeast extract – 10, agar – 15. The culture was run for 48 h at a temperature of 28°C. After that period, colonies grown on plates were counted. The number of yeast cells in the slurry was expressed in  $\text{cfu}/\text{cm}^3$ .

Determination of the viability of yeast cells consisted in the observation of microscopic pictures of cells stained with an aqueous solution of methylene blue in 1:10 000 dilution.

Determination of the content of magnesium in yeast biomass was carried out with the method of atomic absorption spectrophotometry (AAS). Yeast samples were centrifuged at 3500 rpm for 10 minutes (MPW-365, Poland). Supernatant was decanted from above the precipitate and the biomass obtained was dried (SML32/250, ZAIMED, Poland) at the temperature of 105°C to a constant weight. The dried biomass was next mineralized in a mixture of concentrated nitric acid and perchloric acid. The samples were subjected to the mineralization process in a Büchi Digestion Unit K-435 (Germany). The mineralized samples were determined for the content of magnesium in an Atomic Absorption Spectrophotometer (Shimadzu AA-660) at  $\lambda = 285.2 \text{ nm}$ . Determinations were carried out in triplicate after 15 min, 1 h, 2 h, 4 h and 6 h of yeast suspension in a solution of  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ .

The results obtained were subjected to a statistical analysis with the use of Statgraphics Plus ver. 4.1 software. The least significant differences between mean values of the parameters examined during yeast culture were compared with the Tukey's test at a significance level of  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

The content of magnesium in yeast cells (from the slurry collected from the brewery) reached 1.69 mg/g d.m. The optimum concentration of magnesium in the medium indispensable for the proper growth of yeast fluctuates at a level from 10 to 20 mM (0.24–0.48 g/dm<sup>3</sup>) [Walker and Maynard 1996], whereas the minimum demand for magnesium accounts for 1.7 mM (0.041 g/dm<sup>3</sup>) [Rees and Stewart 1997].

**Biosorption of magnesium by yeast depending on the number of cells in yeast slurry**

The process of magnesium biosorption was carried out at a temperature of 28°C. That temperature has been shown to be optimal for the process of magnesium accumulation by yeast cells [Blackwell et al. 1995, Pasternakiewicz and Tuszyński 1997].

Changes in the content of magnesium in yeast cells depending on the variant of yeast slurry preparation applied and the time of its suspension in the MgCl<sub>2</sub>·6H<sub>2</sub>O solution under anaerobic conditions were presented in Table 1.

Table 1. Content of magnesium in biomass of waste brewery yeast suspended in the MgCl<sub>2</sub>·H<sub>2</sub>O solution under anaerobic conditions, mg/g d.m. ±SD

Tabela 1. Zawartość magnezu w biomacie odpadowych drożdży piwowarskich przetrzymywanych w roztworze MgCl<sub>2</sub>·H<sub>2</sub>O w warunkach bez napowietrzania, mg/g s.s. ±SD

Time, h Czas, h	Yeast slurry – Gęstwa drożdżowa			
	control sample próbka kontrolna	10-fold condensed 10-krotnie zagęszczona	2-fold condensed 2-krotnie zagęszczona	2-fold diluted 2-krotnie rozcieńczona
	number of cells, cfu/cm <sup>3</sup> – liczba komórek, jtk/cm <sup>3</sup>			
	5.7 · 10 <sup>8</sup>	9.9 · 10 <sup>9</sup>	1.8 · 10 <sup>9</sup>	3.5 · 10 <sup>8</sup>
0.25		3.18 ±0.42 <sup>b</sup>	4.08 ±0.21 <sup>cd</sup>	7.09 ±0.34 <sup>e</sup>
1		3.36 ±0.44 <sup>bc</sup>	4.29 ±0.16 <sup>cd</sup>	7.24 ±0.71 <sup>e</sup>
2	1.69 ±0.10 <sup>a</sup>	3.02 ±0.13 <sup>b</sup>	4.29 ±0.17 <sup>cd</sup>	7.10 ±0.23 <sup>e</sup>
4		2.98 ±0.26 <sup>b</sup>	4.42 ±0.09 <sup>d</sup>	7.41 ±0.18 <sup>e</sup>
6		3.06 ±0.10 <sup>b</sup>	4.37 ±0.03 <sup>d</sup>	7.63 ±0.53 <sup>e</sup>

SD – standard deviation.

The same letter index denotes a lack of significant difference.

SD – odchylenie standardowe.

Ten sam indeks oznacza brak istotnej różnicy.

The number of yeast cells in the slurry originated from the brewery accounted for 5.7·10<sup>8</sup> cfu/cm<sup>3</sup>. Variant I consisted in tenfold condensation of the slurry. The number of cells in that variant reached 9.9·10<sup>9</sup> cfu/cm<sup>3</sup>, substantially exceeding the initial cell count in the slurry. The content of magnesium absorbed by yeast cells ranged from 2.98 to 3.36 mg/g d.m.

In variant II, the yeast slurry was condensed two times, and the number increased up to 1.8·10<sup>9</sup> cfu/cm<sup>3</sup>. The mean content of magnesium in the biomass ranged then from 4.08 to 4.42 mg/g d.m., which constituted a ca. 2.5-fold increase as compared to the magnesium content in the non-supplemented yeasts and that already after 15 minutes of yeast suspension in a MgCl<sub>2</sub>·6H<sub>2</sub>O solution.

In the next experiment, the yeast slurry was diluted. In variant III, the centrifuged slurry was suspended in a two-fold higher volume of the MgCl<sub>2</sub>·6H<sub>2</sub>O solution in respect of the centrifuged supernatant. The number of cells was observed to decrease by nearly a half, i.e. to a value of 3.5·10<sup>8</sup> cfu/cm<sup>3</sup>. The content of magnesium absorbed by yeast cells was significantly higher than in the experiments carried out with the con-

densed slurry from variants I and II. The mean content of magnesium in yeast (in two-fold diluted slurry) was found to range from 7.09 to 7.63 mg/g d.m. and was ca. 4.3-fold higher than in the cells of the control sample.

Based on the results obtained for the cultures in the experimental variants applied, it was noted that the lower the concentration of cells in the slurry, the higher number of magnesium ions bound with yeast cells. Dilution of the slurry had a more beneficial effect on the biosorption of  $Mg^{2+}$  ions by cells of *S. uvarum* yeasts.

The reported study indicated also that the longer time of slurry suspension in the  $MgCl_2 \cdot 6H_2O$  solution had no significant effect on magnesium biosorption by cells of waste yeasts *S. uvarum* into magnesium ions (Table 1). As early as in the first minutes of the experiment the content of magnesium in yeast cells was increasing significantly as compared to the control sample. The elongated time of the experiment did not cause any significant differences in biosorption of that ion by yeast cells. The results obtained point to the intensive binding of  $Mg^{2+}$  ions with structure of the cell wall of yeasts already in the first minutes of introducing the slurry into the  $MgCl_2 \cdot H_2O$  solution. It should be borne in mind, however, that these ions are adsorbed on the surface of cells. Magnesium, due to a high charge density, may bind with ligands present in the cell wall of yeasts. As a bivalent cation, that ion forms very strong coordinative bonds with free electron pairs occurring in carboxyl and phosphate groups [Walker 1994]. Rapid biosorption of magnesium proceeds also with hydroxyl, amine and bisulfate groups [Pasternakiewicz and Tuszyński 1997]. The initial binding may also proceed through the formation of complexes with extracellular polymers that demonstrate a polysaccharide or glycoprotein structure [Lipke and Ovalle 1998]. The longer suspension of the yeast slurry in the solution containing magnesium contributes to the transfer of its ions from the cell wall to cytosol and creates the possibility of its permanent binding with extracellular structures of yeasts [Błażejczak et al. 2002].

Changes in the content of magnesium in yeast cells depending on the variant of yeast slurry preparation applied and the time of its suspension in the  $MgCl_2 \cdot 6H_2O$  solution under aerobic conditions were presented in Table 2.

As in the case of the previous part of the study, it was observed that the concentrations of the slurry applied had a significant effect on biosorption of magnesium ions by yeast cells. Aeration of yeasts suspended in the hydrated magnesium salt was the least effective from the point of view of magnesium content of biomass – in the case of ten-fold condensation of cells, and the most favourable in variant III, i.e. in the twofold diluted slurry.

A high concentration of yeast cells prepared according to variant I did not evoke any significant increase in magnesium content of cells during 6-hour observation. This resulted, most probably, from the limited access of  $Mg^{2+}$  ions to cells' surface.

At the application of the double diluted number of cells, better binding of that element by yeast was obtained as compared to variant I, which was most likely due to a better access of  $Mg^{2+}$  ions to cell wall of yeasts. The content of magnesium in yeast cells reached 4.51-5.11 mg/g d.m. and increased significantly as compared to yeast cells in the control sample. The time of yeast suspension in the solution of magnesium chloride had no significant effect on the biosorption of that element by cells.

Once the supernatant was substituted with an equal volume of  $MgCl_2 \cdot 6H_2O$  solution, the binding of magnesium was slightly better in variants I and II. In the first hour of the experiment, yeasts bound 4.13 mg Mg/g d.m. In the few subsequent hours, magnesium content remained at a similar level (4.33-4.51 mg/g d.m.).

Table 2. Content of magnesium in biomass of waste brewery yeast suspended in the  $MgCl_2 \cdot H_2O$  solution under aerobic conditions, mg/g d.m.  $\pm$ SDTabela 2. Zawartość magnezu w biomacie odpadowych drożdży piwowskich przetrzymywanych w roztworze  $MgCl_2 \cdot H_2O$  w warunkach z napowietrzaniem, mg/g s.s.  $\pm$ SD

Time, h Czas, h	Yeast slurry – Gęstwa drożdżowa			
	control sample próbka kontrolna	10-fold condensed 10-krotnie zagęszczona	2-fold condensed 2-krotnie zagęszczona	2-fold diluted 2-krotnie rozcieńczona
	number of cells, cfu/cm <sup>3</sup> – liczba komórek, jtk/cm <sup>3</sup>			
	$5.7 \cdot 10^8$	$9.9 \cdot 10^9$	$1.8 \cdot 10^9$	$3.5 \cdot 10^8$
0.25		$2.50 \pm 0.09^b$	$4.51 \pm 0.12^c$	$12.28 \pm 0.22^d$
1		$2.56 \pm 0.06^b$	$4.92 \pm 0.14^c$	$13.00 \pm 0.20^d$
2	$1.69 \pm 0.10^a$	$2.64 \pm 0.02^b$	$5.03 \pm 0.07^c$	$12.89 \pm 0.37^d$
4		$2.72 \pm 0.06^b$	$5.11 \pm 0.10^c$	$12.97 \pm 0.04^d$
6		$2.71 \pm 0.12^b$	$4.97 \pm 0.06^c$	$13.76 \pm 0.19^e$

SD – standard deviation.

The same letter index denotes a lack of significant difference.

SD – odchylenie standardowe.

Ten sam indeks oznacza brak istotnej różnicy.

The best results were obtained after twofold dilution of the slurry with the  $MgCl_2 \cdot 6H_2O$  solution, hence then yeasts were observed to bind 12.28-13.76 mg Mg/g d.m. within 6 hours. The concentration of magnesium increased from 7.3- to 8.1-fold as compared to yeasts from the control sample. Aeration of the slurry contributed to twice as much binding of that element by cell as compared to anaerobic conditions (Tables 1 and 2).

### Magnesium biosorption by yeasts depending on solution pH

Analyses were carried out on the yeast slurry two times diluted with a solution of magnesium chloride. Solutions were kept for 6 hours at a temperature of 28°C under anaerobic and aerobic conditions. Levels of pH adopted in that study were determined based on literature data [Brady and Duncan 1994, Park et al. 2003] and own investigations [Błażejczak et al. 2005].

In the medium with pH 5.5, the yeasts were observed to bind from 7.36 to 9.09 mg Mg/g d.m. under anaerobic condition and from 12.76 to 13.35 mg Mg/g d.m. under aerobic conditions (Table 3). The medium with pH 6.0 appeared to be more favourable for the process of magnesium biosorption by brewery yeasts than that with pH 5.5. In such pH, the content of magnesium in biomass after 4 hours of observation fluctuated from 10.76 to 10.13 mg/g d.m. under anaerobic conditions as well as from 12.56 to 13.84 mg  $Mg^{2+}$ /g d.m. under aerobic conditions. Even better results were obtained in the medium with neutral pH where the amount of magnesium absorbed was the highest as compared to the previous variants. The intensive binding of that element occurred as early as after 15 min of yeast suspension in a solution of magnesium salt (Table 3).

Table 3. Content of magnesium in biomass of waste brewery yeasts depending on pH of the  $MgCl_2 \cdot H_2O$  solution, mg/g d.m.  $\pm$ SD

Tabela 3. Zawartość magnezu w biomacie odpadowych drożdży piwowych w zależności od pH roztworu  $MgCl_2 \cdot H_2O$ , mg/g s.s.  $\pm$ SD

Time, h Czas, h	Mean content of magnesium, mg/g d.m. $\pm$ SD Średnia zawartość magnezu, mg/g s.s. $\pm$ SD		
	pH 5.5	pH 6.0	pH 7.0
Anaerobic conditions – Bez napowietrzania			
0.25	7.36 $\pm$ 0.25 <sup>a</sup>	8.82 $\pm$ 0.52 <sup>b</sup>	12.79 $\pm$ 0.09 <sup>c</sup>
1	8.45 $\pm$ 0.43 <sup>b</sup>	9.13 $\pm$ 0.32 <sup>bc</sup>	13.46 $\pm$ 0.43 <sup>c</sup>
2	9.05 $\pm$ 0.36 <sup>b</sup>	9.33 $\pm$ 0.54 <sup>bc</sup>	15.01 $\pm$ 0.55 <sup>f</sup>
4	9.05 $\pm$ 0.33 <sup>b</sup>	10.76 $\pm$ 0.15 <sup>d</sup>	15.49 $\pm$ 0.31 <sup>f</sup>
6	9.09 $\pm$ 0.13 <sup>bc</sup>	10.13 $\pm$ 0.36 <sup>cd</sup>	15.19 $\pm$ 0.46 <sup>f</sup>
Aerobic conditions – Z napowietrzaniem			
0.25	12.76 $\pm$ 0.12 <sup>A</sup>	12.56 $\pm$ 0.24 <sup>A</sup>	11.69 $\pm$ 0.14 <sup>A</sup>
1	12.91 $\pm$ 0.11 <sup>AB</sup>	12.68 $\pm$ 0.18 <sup>A</sup>	13.24 $\pm$ 0.53 <sup>AB</sup>
2	12.90 $\pm$ 0.03 <sup>A</sup>	13.41 $\pm$ 0.06 <sup>AB</sup>	14.72 $\pm$ 0.06 <sup>AB</sup>
4	13.02 $\pm$ 0.12 <sup>AB</sup>	13.46 $\pm$ 0.30 <sup>AB</sup>	16.88 $\pm$ 0.11 <sup>BC</sup>
6	13.35 $\pm$ 0.21 <sup>AB</sup>	13.84 $\pm$ 0.16 <sup>AB</sup>	17.22 $\pm$ 0.06 <sup>C</sup>

SD – standard deviation.

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Ten sam indeks oznacza brak istotnej różnicy.

After the first hour, yeast from the non-aerated slurry contained 13.46 mg Mg/g d.m., whereas after subsequent hours – from 15.01 to 15.49 mg Mg/g d.m. A similar tendency was observed in the aerated slurry, in which – beginning from the second hour of the experiment – the content of magnesium ranged from 14.72 to 17.22 mg/g d.m.

The results obtained enable concluding that medium pH belongs to important physicochemical factors that affect the process of  $Mg^{2+}$  ions biosorption by cellular structures of yeasts. In the acidic medium, the content of free metal ions increases at concomitant competence of  $[H^+]$  cations with metal cations for chemisorption site on the cell's surface [Tuszyński and Pasternakiewicz 1999]. In the medium with neutral pH, the absorption of metal cations by yeast cells is higher. That phenomenon results from easier formation of links between free electron pairs of ligands of of yeast cell wall and metal ions in the medium with a lower concentration of  $[H^+]$  ions. Definitely unfavourable conditions for ion metals binding by cells occur in the alkaline medium [Park et al. 2003]. High pH values usually contribute to precipitation of sparingly-soluble precipitates of hydroxides and metal oxides, which impairs the binding of magnesium ions with yeasts [Tuszyński and Pasternakiewicz 1999].



## CONCLUSIONS

1. The investigated slurry of *S. uvarum* yeast demonstrated the capacity for magnesium biosorption from a hydrated salt of magnesium chloride.

2. Dilution of the yeast slurry significantly affects the content of magnesium bound with the cells. The highest content of magnesium was obtained in cells from the more diluted slurry, whereas a significantly lower one – from the slurry with a high concentration of cell biomass.

3. The concentration of hydrogen ions (pH) has a significant effect on the biosorption of  $Mg^{2+}$  ions by cells of *S. uvarum* yeasts. An increase in pH value in the range examined (5.5-7.0) evokes a significant increase in the content of magnesium in yeast cells.

## REFERENCES

- Blackwell K.J., Singleton I., Tobin J.M., 1995. Metal cation uptake by yeast: a review. *Appl. Microbiol. Biotechnol.* 43, 579-584.
- Błażej S., Duszkiewicz-Reinhard W., Gniewosz M., Rostkowska-Demmer E., Domurad E., 2002. Badanie zdolności wiązania magnezu przez drożdże piwowarskie *Saccharomyces cerevisiae* w warunkach hodowli stacjonarnej [The study of *Saccharomyces cerevisiae* brewery yeast strain capacity of binding with magnesium in stationary conditions]. *Acta Sci. Pol., Technol. Aliment.* 1, 55-69 [in Polish].
- Błażej S., Duszkiewicz-Reinhard W., Gniewosz M., Chojnicka M., 2005. Wpływ pH na zdolność wiązania magnezu przez drożdże paszowe *Candida utilis* ATCC 9950 podczas hodowli węgłbnej [Influence of the pH factor of the culture medium enriched with magnesium ions on the ability of binding this element by the yeast *Candida utilis* ATCC 9950 in dynamic conditions]. *Acta Sci. Pol., Technol. Aliment.* 4(2), 47-57 [in Polish].
- Brady D., Duncan J., 1994. Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 41, 149-154.
- Brady D., Stoll A.D., Starke L., Duncan J.R., 1994. Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* 44, 297-302.
- Bui M.D., Gregan J., Jarosch E., Ragnini A., Schweyen R.J., 1999. The bacterial magnesium transporter CorA can functionally substitute for its putative homologue Mrs2p in the yeast inner mitochondrial membrane. *J. Biol. Chem.* 274 (29), 20438-20443.
- Emelyanova E.V., 2001. Relationship between magnesium and iron uptake by the yeast *Candida ethanolica*. *Process Biochem.* 36, 517-523.
- Fourest E., Roux J.C., 1992. Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. *Appl. Microbiol. Biotechnol.* 37, 399-403.
- Gardner R.C., 2003. Genes for magnesium transport. *Curr. Opin. Plant Biol.* 6, 263-267.
- Göksungur Y., Üren S., Güvenc U., 2005. Biosorption of cadmium and lead by ethanol treated waste baker's biomass. *Bioresour. Technol.* 96(1), 103-109.
- Graschopf A., Stadler J.A., Hoellerer M.K., Eder S., Sieghardt M., Kohlwein S.D., Schweyen R.J., 2001. The yeast plasma membrane protein Alr1 controls  $Mg^{2+}$  homeostasis and is subject to  $Mg^{2+}$  – dependent control of its synthesis and degradation. *J. Biol. Chem.* 276, 16216-16222.
- Gregan J., Kolisek M., Schweyen R.J., 2001. Mitochondrial  $Mg^{2+}$  homeostasis is critical for group II intron splicing in vivo. *Genes Dev.* 15, 2229-2237.
- Hiromura M., Sakurai H., 2001. Intracellular metal transport protein. *Riken Rev.* 35, 23-25.

- Kapteyn J.C., Hoyer L.L., Hecht J.E., Muller W.H., Andel A., Verkleij A.J., Makarow M., Van den Ende H., Klis F.M., 2000. The cell wall architecture of *Candida albicans* wild-type cells and cell wall-defective mutants. *Mol. Microbiol.* 35, 601-611.
- Klis F.M., Mol P., Hellingwerf K., Brul S., 2002. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* 26, 239-256.
- Knoop V., Groth-Malonek M., Gebert M., Eifler K., Weyand K., 2005. Transport of magnesium and other divalent cations: evolution of the 2-TM-GxN proteins in the MIT superfamily. *Mol. Genet. Genomics* 274 (3), 205-216.
- Liu G.J., Martin D.K., Gardner R.C., Ryan P.R., 2002. Large  $Mg^{2+}$  – dependent currents are associated with the increased expression of ALR1 in *Saccharomyces cerevisiae*. *Microbiol. Lett.* 213 (2), 231-237.
- Lipke P.N., Ovalle R., 1998. Cell wall architecture in yeast: new structure and new challenges. *J. Bacteriol.* 180, 3735-3740.
- Lo W., Chua H., Lam K.H., 1999. A comparative investigation on the biosorption of lead by filamentous fungal biomass. *Chemosphere* 39, 2723-2736.
- MacDiarmid C.W., Gardner R.C., 1998. Overexpression of the *Saccharomyces cerevisiae* magnesium transport system confers resistance to aluminum ion. *J. Biol. Chem.* 273, 1727-1732.
- Orlean P., 1997. Biogenesis of yeast wall and surface components. In: *Molecular and cellular biology of the yeast Saccharomyces*. Eds J. Pringle, J. Broach, E. Jones. Cold Spring Harbor Lab. Press, Cold Spring Harbor New York, 229-362.
- Park J.K., Lee J.W., Jung J.Y., 2003. Cadmium uptake capacity of two strains of *Saccharomyces cerevisiae* cells. *Enzyme Microbiol. Technol.* 33(4), 371-378.
- Pasternakiewicz A., Tuszyński T., 1997. Effect of calcium, magnesium, cobalt (II<sup>+</sup>), and zinc cations on the *Saccharomyces cerevisiae* growth. *Pol. J. Food Nutr. Sci.* 47(4), 61-70.
- Rees M., Stewart G., 1997. The effects of increased magnesium and calcium concentration on yeast fermentation performance in high gravity worts. *J. Inst. Brew.* 103, 287-291.
- Saltukoglu A., Slaughter J.C., 1983. The effect of magnesium and calcium on yeast growth. *J. Inst. Brew.* 89, 81-83.
- Słaba M., Długoński J., 2002. Mikrobiologiczne odzyskiwanie i usuwanie metali ciężkich [Microbiological heavy metals recovery and removal]. *Post. Mikrobiol.* 41, 2, 167-183 [in Polish].
- Thomas K.C., 1980. Effect of pH on the rate of *Candida utilis* cell cycle initiation. *J. Bacteriol.* 144(3), 1193-1196.
- Tuszyński T., Pasternakiewicz A., 1999. Oddziaływanie jonów metali na wzrost drożdży *Saccharomyces cerevisiae* w zależności od pH podłoża hodowlanego [Interaction of metal ions on the yeast growth *Saccharomyces cerevisiae* in depending of medium pH]. *Chem. Inż. Ekol.* 6(5-6), 720-728 [in Polish].
- Tuszyński T., Pasternakiewicz A., 2000. Bioaccumulation of metal ions by yeast cell of *Saccharomyces cerevisiae*. *Pol. J. Food Nutr. Sci.* 4, 31-39.
- Vaart Van der J.M., Caro L.H.P., Chapman J.W., Klis F.M., Verrips C.T., 1995. Identification of three mannoproteins in cell wall of *Saccharomyces cerevisiae*. *J. Bacteriol.* 177, 3104-3110.
- Walker G., 1994. The roles of magnesium in biotechnology. *Crit. Rev. Biotechnol.* 14(4), 311-354.
- Walker G.M., Maynard A.I., 1996. Magnesium-limited growth of *Saccharomyces cerevisiae*. *Enzyme Microb. Technol.* 18, 455-459.
- White C., Gadd G.M., 1987. The uptake and cellular distribution of zinc in *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* 133, 727-737.

**BADANIA BIOSORPCJI MAGNEZU PRZEZ ODPADOWE DROŹDŹE  
PIWOWARSKIE *SACCHAROMYCES UVARUM***

**Streszczenie.** Badano zdolność odpadowych drożdży piwowskich *Saccharomyces uvarum* do biosorpcji magnezu pochodzącego z roztworu uwodnionej soli chlorku magnezu, w zależności od liczby komórek i zróżnicowanego pH zawiesiny w czasie 6 h. Zawartość  $MgCl_2 \cdot 6H_2O$  w roztworze regulowano tak, aby zachować stałą zawartość magnezu w przeliczeniu na czysty pierwiastek, tj. 1,25 g Mg na  $1\text{ dm}^3$  roztworu. W pierwszym etapie różnicowano liczbę komórek w gęstwie drożdżowej przez zagęszczanie lub rozcieńczanie. W drugim etapie różnicowano pH zawiesiny drożdży (pH 5,5, 6,0 i 7,0) przy stałej liczbie komórek. Badane roztwory przetrzymywano w warunkach bez napowietrzania i z napowietrzaniem. Oznaczenie zawartości magnezu w biomacie drożdży wykonywano metodą atomowej spektroskopii adsorpcyjnej po 15 min, 1 h, 2 h, 4 h i 6 h doświadczeń. Największą zawartość magnezu (13,76 mg/g s.s.) uzyskano z najmniejszą liczbą komórek w roztworze,  $3,5 \cdot 10^8/\text{cm}^3$ , w warunkach z napowietrzaniem. Wzrost pH roztworu sprzyjał biosorpcji magnezu przez drożdże. W pH = 7,0 po 6 h drożdże zawierały magnez w wysokości 15,19 mg/g s.s. w warunkach bez napowietrzania oraz 17,22 mg/g s.s. w warunkach z napowietrzaniem.

**Słowa kluczowe:** magnez, drożdże piwowskie, *Saccharomyces uvarum*

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