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STUDIES INTO *SACCHAROMYCES CEREVISIAE* BAKER'S YEAST CAPACITY FOR BINDING MAGNESIUM UNDER BATCH CONDITIONS

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The capacity for natural binding of magnesium by baker's yeast (*S. cerevisiae* No. 102) was investigated under batch conditions (with aeration) on control medium (YPD) and experimental media enriched with Mg^{2+} ions at the following doses: 0.25 g/L; 0.5 g/L and 1.25 g/L. The source of Mg^{2+} ions were magnesium salts: $MgSO_4 \cdot 7H_2O$ or $MgCl_2 \cdot 6H_2O$, which were added to the culture media at the beginning of incubation and at the end of the logarithmic growth phase of yeasts. Magnesium content was determined with the AAS method in non rinsed biomass (magnesium not permanently bound with cells) and biomass rinsed twice with deionised water (magnesium permanently bound with cells). There was more magnesium not permanently bound with yeast cells. The medium enriched with chloride salt was found to bind more magnesium ions than the medium supplemented with sulphate salt. The content of magnesium permanently bound with cell biomass obtained after 6-h incubation with the addition of chloride salt (3.50–3.71 mg Mg/g d.s.) was twice as high as the content of magnesium bound with the biomass from control medium. The applied concentrations of Mg^{2+} ions had no significant effect on the content of magnesium permanently bound with cell yeasts. Magnesium addition to media at the end of the logarithmic growth phase was found not to increase magnesium content in the yeast cell biomass after 24 h and 48 h of incubation. The addition of magnesium chloride to YPD medium intensified considerably the growth rate between the 6^{th} and 24^{th} h of culture and had a positive effect on the final yield of yeast cell biomass.

INTRODUCTION

One of the more important elements occurring in living organisms is magnesium. In a human body this element serves multiple functions: it activates a number of enzymes, forms tissues, plays an important function in the physiology of the nervous, muscular, cardiac, osteoarticular, respiratory, alimentary and haematopoietic systems [Walasek, 1998]. Statistical surveys indicate that nearly 50% of the Polish population suffers from magnesium deficiency, which is reflected in deteriorated functioning of the organism. Major symptoms of magnesium deficiency include: contractions and weakness of muscles, convulsions, irritation, nervousness, physical and mental fatigue [Pasternak, 1999; Brzozowska, 1998].

Bearing in mind that as little as 30–50% of magnesium is absorbed with food, there is a need of its constant supplementation in a diet. Magnesium may be supplemented in two types of preparations: non-organic and organic magnesium salts or the so-called metalloproteins, *i.e.* magnesium complexes with proteins. The latter are characterised by increased availability by human and animal organisms, compared to non-organic and organic magnesium salts [Krejpcio *et al.*, 1999; Olędzka, 1999].

One of the ways to obtain metalloproteins (also referred to as bioplexes) involves the enrichment of yeast biomass with that element, and then the production of protein preparations. Yeasts *Saccharomyces cerevisiae* display the capacity for natural binding of magnesium at levels significantly exceeding their physiological demand [Walker & Maynard, 1996; Soral-Śmietana *et al.*, 1999; Tuszyński & Pasternakiewicz, 2000; Błażejak *et al.*, 2002]. In addition, they are commonly known as microorganisms completely safe for humans and animals. Their physiology has been well recognised and the production of baker's yeasts has been applied at the industrial scale for years.

The *S. cerevisiae* No. 102 strain of baker's yeast is known to bind well Mg^{2+} ions from YPD medium enriched with non-organic salts of this element under stationary conditions [Duszkiewicz-Reinhard *et al.*, 2002]. In this study, the highest amounts of magnesium were bound by yeast in the 6^{th} hour of incubation, *i.e.* in the logarithmic growth phase. Under experimental conditions applied therein, the biomass obtained was characterised by an increased content of permanently bound magnesium. Biomass yield recorded under stationary culture conditions (without aeration) was extremely low (*ca.* 3.5 g d.s./L). Thus, the presented study aimed at estimating whether magnesium binding is also so intensive under batch conditions (with aeration) enhancing rapid yeast multiplication.

The objective of this study was to determine the capacity of baker's yeast *S. cerevisiae* (strain No. 102) for natural binding of magnesium depending on the type of magnesium salt, magnesium concentration in the medium and the time

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of medium supplementation under batch conditions (with aeration).

MATERIAL AND METHODS

The experimental material included *Saccharomyces cerevisiae* No. 102 strain of baker's yeasts originating from the Museum of Pure Cultures, Chair of Food Biotechnology and Microbiology, Warsaw Agricultural University. Strains were stored on wort slants [Burbianka & Pliszka, 1983] under cooling conditions.

Inoculum. Inoculum was prepared on liquid YPD medium [Suizu *et al.*, 1996] and multiplied for 24 h at a temperature of 28°C on a laboratory shaker (ROSI 1000, Thermolyne, USA) at 200 rpm until optical density of $OD_{600}=2.1$, which corresponded to the cell number of 1.3×10^7 cfu/mL.

Media. Control medium was the YPD medium, whereas experimental media included the YPD medium enriched with magnesium ions originated from two salts: $MgSO_4\cdot7H_2O$ or $MgCl_2\cdot6H_2O$. Three levels of magnesium salt addition were accepted so that the magnesium content of the media accounted for: 0.25 g Mg^{2+}/L, 0.5 g Mg^{2+}/L, and 1.25 g Mg^{2+}/L of medium. Water solutions of magnesium salts were added to culture medium at the beginning of incubation (variant I) and at the end of the logarithmic growth phase (verified experimentally – own sources), *i.e.* after 18-h incubation (variant II). All media and salt solutions were prepared on deionised water.

Yeast cultures. The control and experimental media were inoculated with inoculum at a dose of 10% (v/v). Yeast cultures (100 mL) were run under batch conditions (with aeration) at a temperature of 28°C for 48 h on a laboratory shaker (ROSI 1000, Thermolyne, USA) at 200 rpm.

The content of cell yeast biomass (biomass yield) was determined after centrifugation of 50 mL of the culture at 3500 rpm for 10 min (MPW 365, Mechanika Precyzyjna, Poland). The wet biomass obtained was dried initially at a temperature of 60°C for 2 h, and then re-dried at 105°C till the constant weight. Determinations were carried out in triplicate. Biomass yield was monitored after 6-, 24- and 48-h incubation (in variant I) and after 24- and 48-h incubation (in variant II). Results were expressed in g d.s./L medium.

Magnesium content in yeast cell biomass was determined after 6-, 24- and 48-h incubation in control and experimental media (in variant I) and after 18-, 24- and 48 h (in variant II). Centrifuged, dried and weighed sample of yeast biomass (*ca.* 0.1 g d.s.) from particular cultures was mineralized, by combustion in a mixture of nitric and perichloric acids added in the amounts of 5 mL and 2 mL, respectively. Mineralization was carried out in a Büchii Digestion Unit K-435 apparatus (Germany). So prepared samples were determined for the content of magnesium with the use of atomic absorption spectrophotometry (AAS, Schimadzu AA660 spectrophotometer, Japan) [Bryłka *et al.*, 1995]. Absorbance was read at $\lambda=285.2$ nm. Results were expressed in mg Mg/g d.s.

The results obtained were analysed statistically. Two-way analysis of variance was carried out with Duncan's test to determine the significance of differences ($p=0.05$). Use was made of Statgraphics Plus ver. 4.1 software.

RESULTS AND DISCUSSION

Determination of yeast cell biomass yield in control and experimental media

Tables 1 and 2 present the yield of *S. cerevisiae* No. 102 yeast biomass incubated on control YPD medium and experimental media supplemented with magnesium ions originated from hydrated magnesium chloride or hydrated magnesium sulfate. Magnesium salts were added to the experimental media at the onset of culture (time t=0). Biomass yield was determined after 6-, 24- and 48-h incubation.

TABLE 1. Biomass yield (g d.s./L) of yeast incubated on control and experimental media (magnesium source: $MgCl_2\cdot6H_2O$ added at the beginning of incubation).

Medium	Time of incubation (h)		
	6	24	48
YPD control	3.90^{a*}	10.0^{b}	11.53^{c}
YPD+0.25 g Mg^{2+}/L	4.60^{a}	14.05^{d}	13.76^{d}
YPD+0.5 g Mg^{2+}/L	4.79^{a}	13.91^{d}	14.09^{d}
YPD+1.25 g Mg^{2+}/L	4.78^{a}	13.66^{d}	13.48^{d}

* – the same letters denote a lack of statistically significant difference at p=0.05

TABLE 2. Biomass yield (g d.s./L) of yeast incubated on control and experimental media (magnesium source: $MgSO_4\cdot7H_2O$ added at the beginning of incubation).

Medium	Time of incubation (h)		
	6	24	48
YPD control	3.90^{a*}	10.0^{b}	11.53^{c}
YPD+0.25 g Mg^{2+}/L	4.51^{a}	9.05^{d}	12.40^{f}
YPD+0.5 g Mg^{2+}/L	3.96^{a}	8.04^{e}	12.45^{f}
YPD+1.25 g Mg^{2+}/L	4.40^{a}	7.49^{e}	12.40^{f}

* – the same letters denote a lack of statistically significant difference at p=0.05

During 48-h culture under batch conditions (with aeration), the control medium was observed to be characterised by intensive growth of cell biomass of baker's yeast. After 6-h incubation, the biomass yield reached 3.90 g d.s./L on average. During the subsequent hours, the yeasts were in the phase of logarithmic growth, when cells are subject to intensive division and their number increases in a geometrical progression. After 24-h incubation, the average yield accounted for 10.0 g d.s./L of medium. After the next 24 h, the biomass yield increased only to 11.53 g d.s./L of medium, which indicates a decline in the growth rate of cells and transition of the population into the stationary phase.

The effect of magnesium ions on the growth of bakery's yeast population in particular days of culture was presented in Tables 1 and 2.

In the first six hours of yeast culture on medium supplemented with $MgCl_2\cdot6H_2O$ no significant effect of Mg^{2+} ions

on the intensity of yeast cell growth was observed (Table 1). Beneficial effect of these ions was reported between 6 and 24 h of incubation. This period was characterised by significantly higher yields of cell biomass obtained from experimental media compared to the biomass yield determined in the control medium. The highest yield of yeast biomass was reported for the medium with the addition of 0.25 g Mg^{2+}/L (Table 1). In this time interval, however, particular doses of Mg^{2+} ions were observed not to have any significant effect on biomass yield which ranged from 13.66 to 14.05 g d.s./L medium. The results obtained indicate that the addition of 0.25–1.25 g Mg^{2+}/L (as hydrated sodium chloride) to medium considerably intensified the growth of yeast cells in the first 24 h of culture. After the second 24-h period, no significant increase in biomass yield was observed in the media with different doses of chloride salt. The biomass yield in these media was reported to range from 13.48 to 14.09 g d.s./L medium.

Other, less beneficial, effect of magnesium ions on biomass yield of baker's yeast was observed upon the addition of sulfate salt to medium, which – as in the case of chloride salt-supplemented medium – did not diversify significantly the biomass yield within the first 6 h, irrespective of the dose applied. After 24 h, a significant decrease in yeast biomass yield was noted, compared to the control medium. Thus, salt addition contributed to a slower growth of yeast cells, which in turn resulted in a diminished biomass yield compared to that determined in the control medium. In addition, it was noticed that the higher the dose of the sulfate salt applied, the lower the biomass yield. In the medium supplemented with 1.25 g Mg^{2+}/L, biomass yield accounted for as little as 7.49 g d.s./L medium. In the second 24 h of culture, biomass yield of yeast cells was observed to further increase, which resulted in significantly higher yeast biomass yields compared to the first 24 h. Consequently, after 48 h the yield of yeast biomass raised up to 12.40–12.76 g d.s./L and was significantly different from that of the control medium.

The result obtained in this study imply that the source of magnesium introduced to the medium exerts a decisive effect on the growth rate of cell biomass and to a lesser extent affects the final yield of yeast cultured under the experimental conditions applied. Despite the same dose of magnesium ions supplemented to experimental media, biomass yield determined under identical culture conditions was significantly different. The greatest, nearly twofold, differences were observed between yeast biomass yields after 24-h culture with the highest dose of magnesium ions (1.25 g Mg^{2+}/L).

A similar tendency was observed by Walker and Maynard [1996] who investigated the effect of Mg^{2+} ions, applied at a dose of 496–13 μmol/L (corresponding to 0.012–0.003 g Mg^{2+}/L), on the growth and metabolism of *Saccharomyces cerevisiae* cells. In this experiment the maximal rate of cell growth was noted at the highest concentration of Mg^{2+} ions in the medium. A lowered concentration of these ions in the medium inhibited, to a great extent, the rate of cell growth, yet it had no significant effect on the final yield of cell biomass. The biomass yield was observed to increase proportionally only up to some boundary value of Mg^{2+} ions concentration in the medium (*ca.* 100 μmol/L, which corresponds to 0.0024 g Mg^{2+}/L). As soon as this value has been exceeded, biomass yield assumed constant values and remained at the same level, irrespective of an increasing concentration of magnesium ions in the medium.

Determination of magnesium content in cell biomass of yeasts cultured on control and experimental media

The capability of the natural binding of magnesium ions by the baker's yeast strain *S. cerevisiae* was investigated in model YPD medium supplemented with non-organic magnesium salts: $MgCl_2 \cdot 6H_2O$ or $MgSO_4 \cdot 7H_2O$. Determinations of magnesium content were carried out after 6, 24 and 48 h of incubation in variant I, and after 18, 24 and 48 h in variant II (Tables 3–6).

TABLE 3. Magnesium content (mg d.s./L) of yeast cell biomass incubated on control and experimental media ($MgCl_2 \cdot 6H_2O$ added at the beginning of incubation).

Medium	Time of incubation (h)					
	6	24	48	6	24	48
	non-rinsed biomass			rinsed biomass		
YPD control	1.90^{a*}	1.67^{a}	1.44^{a}	1.75^{ab}	1.46^{a}	1.25^{a}
YPD+0.25 g Mg^{2+}/L	2.51^{bc}	2.77^{cd}	2.96^{bcd}	3.65^{e}	2.32^{bcd}	2.08^{bc}
YPD+0.5 g Mg^{2+}/L	4.12^{e}	3.51^{de}	3.22^{cd}	3.50^{e}	2.10^{bc}	2.30^{bcd}
YPD+1.25 g Mg^{2+}/L	5.42^{g}	5.58^{g}	5.34^{g}	3.71^{e}	2.70^{d}	2.50^{cd}

* – the same letters denote a lack of statistically significant difference at p=0.05

TABLE 4. Magnesium content (mg d.s./L) of yeast cell biomass incubated on control and experimental media ($MgSO_4 \cdot 7H_2O$ added at the beginning of incubation).

Medium	Time of incubation (h)					
	6	24	48	6	24	48
	non-rinsed biomass			rinsed biomass		
YPD control	1.90^{ac*}	1.67^{ab}	1.44^{ab}	1.75^{abc}	1.46^{ab}	1.25^{a}
YPD+0.25 g Mg^{2+}/L	3.65^{e}	2.48^{bcd}	2.02^{abc}	3.09^{f}	2.05^{cde}	1.94^{bcd}
YPD+0.5 g Mg^{2+}/L	3.63^{e}	3.17^{de}	2.65^{cd}	2.95^{f}	2.14^{cde}	2.22^{de}
YPD+1.25 g Mg^{2+}/L	6.85^{g}	5.62^{f}	5.03^{f}	2.98^{f}	2.35^{e}	2.30^{e}

* – the same letters denote a lack of statistically significant difference at p=0.05

Tables 3 and 4 compile contents of magnesium bound with cell biomass of yeast cultured on control medium and experimental media supplemented with magnesium salts at the beginning of incubation. In non-rinsed yeast biomass obtained from the control medium the average magnesium content reached 1.90 mg Mg/g d.s. after 6 h of culture. In the subsequent hours, its content was observed to decrease to 1.67 mg Mg/g d.s. after 24 h and to 1.44 mg Mg/g d.s. after 48 h of culture. The rinsed yeast biomass was characterised by insignificantly lower contents of magnesium (from 1.75 mg Mg/g d.s. to 1.46 mg Mg/g d.s.) with a similar tendency of a slight decrease in the subsequent hours of culture (Table 3).

Usually, the non-rinsed biomass from experimental media was found to bind more Mg^{2+} ions when their concentration in the medium was higher. The only exception was biomass from the medium containing 0.5 g Mg^{2+} ions/L originating from $MgSO_4 \cdot 7H_2O$. The highest content of magnesium – 6.85 mg Mg/g d.s. – was reported after 6 h of culture in the medium containing 1.25 g Mg^{2+}/L in the form of hydrated magnesium sulfate (Table 4). A lower magnesium content was determined in biomass obtained from the medium containing the same number of Mg^{2+} ions in the form of chloride salt (Table 3). In the consecutive hours of incubation, no significant increase in magnesium content was observed. On the contrary, the content of magnesium in the non-rinsed biomass was observed to decline with time. This tendency was observed irrespective of the source of magnesium present in the medium.

In the rinsed yeast biomass (as in the case of the non-rinsed one), the highest number of Mg^{2+} ions were bound with yeast cells also after 6 h of culture, still their numbers were significantly lower. In this case, more magnesium bound with yeast biomass originated from chloride salt than from sulfate salt. After 6 h of culture, yeast biomass obtained from the media with hydrated magnesium chloride contained 3.50–3.71 mg Mg/g d.s. (Table 3), whereas that originated from the media with hydrated magnesium sulfate – as little as 2.95–3.09 mg Mg/g d.s. (Table 4). Despite increasing concentrations of Mg^{2+} ions in the media, no significant differences were observed in magnesium content of yeast biomass, contrary to the non-rinsed biomass. After 24 h of culture, the contents of magnesium permanently bound with the biomass appeared to decrease significantly, ranging from 2.10 to 2.70 mg Mg/g d.s. in the medium with $MgCl_2 \cdot 6H_2O$, and from 2.05 to 2.35 mg Mg/g d.s. in the medium with $MgSO_4 \cdot 7H_2O$). In the next 24 h of culture (*i.e.* after 48 h), the level of that element was not subject to any significant changes.

These observations clearly indicate that during natural growth yeasts are able to bind permanently only some specified amount of magnesium. The excess of this element appears outside yeast cells and in the subsequent hours of culture does not penetrate into their interior. The higher the number of magnesium ions added to medium, the higher the amount of ions not permanently bound with yeast cell wall. This fact may be explained by a high affinity of divalent metal cations to negatively-charged protein-carbohydrate complexes present in the cell wall [Gardner, 2003; Chmiel, 1998; Walker, 1994]. These complexes contain carboxyl, hydroxyl, phosphate, amine, and other groups which occur in phosphomannates and the outer, manno-protein layer of yeast cell wall [Brady & Duncan, 1994; Brady *et al.*, 1994; Lipke & Ovalle, 1998; Lo *et al.*, 1999]. Still, the adsorption of Mg^{2+} ions with the cell wall is not too strong, hence magnesium is easily removed during biomass washing with deionised water.

The process of magnesium adsorption to a cell wall does not require any energy expenditure by the cell, compared to the bioaccumulation process proceeding in a much slower manner and connected with active transport of magnesium through the cell wall and cytoplasmatic membrane into the cell's interior [Blackwell *et al.*, 1995]. This part of Mg^{2+} ions actively participates in nearly all metabolic processes of a cell, exerts a stabilising effect on genetic material and acts as a co-factor of almost all enzymes [Hartwing, 2001]. In the cell magnesium is bound with intracellular proteins, including: transport, enzymatic or structural ones. Some of them have already been recognised, *i.a.* transport proteins Alr1p and Alr2p (belonging to the family of membrane integral proteins - MIT) present in cytoplasmatic membrane which participate in magnesium transport to cytosole [Graschopf *et al.*, 2001], or the so-called metallothioneins – low-molecular-weight proteins with a high content of cystein [Truchliński & Pasternak, 2002]. The role of magnesium in the bond with enzymatic proteins consists in their activation (*e.g.* DNA polymerase or protein kinase A) [Stryer, 1997; Cohen, 1998], which enables the stimulation of cell growth and the extension of their vitality. It should be borne in mind, however, that despite a peculiar effect of magnesium on cell growth, the accumulation of unlimited amounts of magnesium in cytose is impossible. Thus, yeast cells are able to bind magnesium permanently only at some constant level, above which further bioaccumulation inside the cell is not possible. The threshold value of such bioaccumulation is likely to be a strain-specific trait of yeast. Some excess of magnesium may be accumulated by yeast inside vacuoles, mainly in the form of polyphosphate complexes [Brady & Duncan, 1994].

Under experimental conditions applied in this study, the yeast biomass obtained was characterised by nearly twofold higher content of magnesium permanently bound with the biomass, compared to the control medium, and it seems that these values were boundary to the yeast strain examined.

Tables 5 and 6 present contents of magnesium in yeast biomass obtained from experimental media supplemented with magnesium salts at the end of the logarithmic phase of growth, *i.e.* after 18-h culture (variant II). This experiment aimed at comparing magnesium bioaccumulation in the population of mature, well-nourished cells multiplying to a lesser extent with that in the population of cells gemmating intensively from the logarithmic phase of growth. An attempt was also made to eliminate the negative effect of high concentrations of Mg^{2+} ions applied in the form of sulfate salt on the growth rate of yeast (Table 2), which was likely to affect weaker binding of magnesium by biomass within the first 6 h of yeast culture.

In the non-rinsed biomass cultured in the medium with the addition of chloride salt the content of magnesium was

TABLE 5. Magnesium content (mg d.s./L) of yeast cell biomass incubated on control and experimental media ($MgCl_2 \cdot 6H_2O$ added at the end of logarithmic growth phase).

Medium	Time of incubation (h)					
	18	24	48	18	24	48
	non-rinsed biomass			rinsed biomass		
YPD control	1.80^{abc*}	1.67^{ab}	1.44^{a}	1.63^{ab}	1.46^{a}	1.25^{a}
YPD+0.25 g Mg^{2+}/L	2.58^{def}	2.47^{cde}	2.25^{bcd}	2.10^{c}	1.88^{bcd}	1.56^{ab}
YPD+0.5 g Mg^{2+}/L	3.18^{ef}	3.31^{f}	2.97^{def}	2.27^{cd}	2.10^{cd}	2.05^{cd}
YPD+1.25 g Mg^{2+}/L	5.83^{h}	5.36^{gh}	4.82^{g}	2.30^{d}	2.82^{e}	2.28^{cd}

* – the same letters denote a lack of statistically significant difference at p=0.05

TABLE 6. Magnesium content (mg d.s./L) of yeast cell biomass on control and experimental media ($MgSO_4 \cdot 7H_2O$ added at the end of incubation).

Medium	Time of incubation (h)					
	18	24	48	18	24	48
	non-rinsed biomass			rinsed biomass		
YPD control	1.80^{abc*}	1.67^{ab}	1.44^{a}	1.63^{ab}	1.46^{a}	1.25^{a}
YPD+0.25 g Mg^{2+}/L	2.59^{cde}	2.37^{bcd}	2.08^{abc}	2.12^{def}	2.41^{fg}	1.84^{bcd}
YPD+0.5 g Mg^{2+}/L	3.03^{de}	3.24^{e}	3.03^{de}	2.38^{efg}	2.48^{fg}	2.00^{cde}
YPD+1.25 g Mg^{2+}/L	5.66^{g}	4.97^{fg}	4.88^{f}	2.42^{fg}	2.51^{g}	2.30^{efg}

* – the same letters denote a lack of statistically significant difference at p=0.05

at a similar level as in the case of biomass obtained from the medium supplemented with this salt at the beginning of culture (variant I), (Tables 3 and 5). Significant differences were observed only between the contents of magnesium permanently bound with biomass. After 18-h culture, magnesium binding by the population with the dominating number of mature, rarely gemmating cells (after medium supplementation with magnesium chloride) was observed not to be more intensive than by the cells from the logarithmic phase of growth. In the 18th h of culture, magnesium content in the biomass was significantly lower than in the biomass obtained after 6 h of culture in variant I. After 24 h, the contents of magnesium in the biomass were increasing along with the increasing concentration of Mg^{2+} ions in the medium and ranged from 1.88 mg Mg/g d.s. to 2.82 mg Mg/g d.s. After 48 h of culture, its content was observed to drop insignificantly to 1.56–2.28 mg Mg/g d.s. The lowest amount of magnesium was bound with the biomass incubated with the addition of 0.25 g Mg^{2+}/L.

The addition of sulfate salt to the medium at the end of the logarithmic growth phase appeared less beneficial than its addition at the beginning of culture (variant I), (Tables 4 and 6). Although the level of magnesium in the non-rinsed biomass was raising with the increasing concentration of $MgSO_4 \cdot 7H_2O$ in the medium, its content was lower than that in the biomass from variant I, especially in the medium with the addition of 1.25 g Mg^{2+}/L (Table 6.). After 24-h culture, the content of magnesium not permanently bound with the biomass ranged from 2.37 to 4.97 mg Mg/g d.s, and after the 48th h it remained at a similar level, *i.e.* 2.08–4.88 mg Mg/g d.s. In the rinsed biomass, after 24-h culture, the levels of magnesium were almost alike (2.41–2.51 mg Mg/g d.s.), still they were not significantly different for different concentrations of Mg^{2+} ions in the medium. In the next 24 h of culture, a slight drop in magnesium content of biomass was noted, as in variant I (Table 4 and 6).

A statistical analysis of the results obtained demonstrated a significant reciprocal effect of the variables examined (time of culture and Mg^{2+} dose in the medium) on biomass yield and magnesium content of yeast cells.

The result of this study indicate that a high yield of magnesium-enriched biomass was obtained in the first 24 h of culture and that permanent binding of magnesium proceeds with a greater intensity in multiplying cell (6–24 h of culture) than in the older cells whose population transits into the stationary phase.

CONCLUSIONS

1. Under batch culture conditions applied (with aeration), the highest content of magnesium bound with the non-rinsed biomass (6.85 mg Mg/g d.s.) was obtained after 6 h in the medium supplemented with 1.25 g Mg^{2+}/L in the form of sulfate salt. Presumably, a greater part of magnesium was bound with yeast cell wall not permanently.

2. At the beginning of culture (after 6 h), in the media enriched with $MgCl_2 \cdot 6H_2O$ yeasts were observed to bind permanently from 3.50 to 3.71 mg Mg/g d.s. Irrespective of magnesium concentration examined, cells of these yeast appeared to bind twice as much magnesium as these from the control medium.

3. The addition of magnesium salts at the end of the logarithmic phase of growth was demonstrated to exert a smaller effect on magnesium binding by yeast cells compared to their addition at the beginning of culture. After 18 h, the content of permanently bound magnesium fluctuated from 2.10 to 2.42 mg Mg/g d.s.

4. The enrichment of culture medium with $MgCl_2 \cdot 6H_2O$ (irrespective of the dose applied) affected beneficially the growth rate of yeast population between the 6 and 24 h of culture, and – to a smaller extent – the final yield of cell biomass.

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BADANIA ZDOLNOŚCI WIĄZANIA MAGNEZU PRZEZ DROŻDŻE PIEKARSKIE *SACCHAROMYCES CEREVISIAE* W WARUNKACH HODOWLI WGŁĘBNEJ

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Badano zdolność naturalnego wiązania magnezu przez drożdże piekarskie (szczep Nr 102) podczas hodowli wgłębnej (z napowietrzaniem) na podłożu kontrolnym (YPD) i podłożach doświadczalnych, tj. YPD wzbogaconym w jony Mg^{2+} w ilości 0,25 g/L; 0,5 g/L i 1,25 g/L. Źródłem jonów Mg^{2+} były dwie sole: $MgSO_4 \cdot 7H_2O$ lub $MgCl_2 \cdot 6H_2O$. Sole magnezu dodawano do podłoży na początku hodowli oraz pod koniec logarytmicznej fazy wzrostu drożdży. Zawartość magnezu oznaczano metodą ASA w biomasie nie przemywanej oraz w biomasie dwukrotnie przemywanej wodą dejonizowaną. Większa część magnezu była nietrwale związana z komórkami drożdży, co stwierdzono przez porównanie zawartości magnezu w biomasie przemywanej z zawartością tego pierwiastka w biomasie nieprzemywanej. W podłożu z dodatkiem soli chlorkowej biomasa komórkowa związała więcej magnezu niż w podłożu z dodatkiem soli siarczanowej. Zawartości magnezu trwale związanego z biomasą komórkową otrzymaną po 6-godzinnej hodowli z dodatkiem soli chlorkowej (3.50–3.71 mg Mg/g s.s.) dwukrotnie przewyższały zawartość magnezu związanego z biomasą otrzymaną z podłoża kontrolnego. Zastosowane stężenia jonów Mg^{2+} nie miały istotnego wpływu na zawartość magnezu trwale związanego z biomasą. Dodatek magnezu do podłoży pod koniec logarytmicznej fazy wzrostu nie spowodował podwyższenia zawartości magnezu w biomasie badanej po 24 i 48 godzinach hodowli. Dodatek chlorku magnezu do podłoża YPD znacznie zintensyfikował tempo wzrostu populacji między 6 a 24 godziną hodowli, jak również korzystnie wpłynął na końcowy plon biomasy komórkowej drożdży.