Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2002
Volume 5
Issue 2
**Series FOOD SCIENCE AND TECHNOLOGY**


BŁAŻEJAK S., DUSZKIEWICZ-REINHARD W., GNIEWOSZ M., ROSTKOWSKA-DEMNER E., DOMURAD E. 2002. THE STUDY OF *SACCHAROMYCES CEREVISIAE* BREWERY YEAST STRAIN CAPACITY OF BINDING WITH MAGNESIUM IN DYNAMIC CONDITIONS **Electronic Journal of Polish Agricultural Universities**, Food Science and Technology, Volume 5, Issue 2.
Available Online http://www.ejpau.media.pl

# THE STUDY OF *SACCHAROMYCES CEREVISIAE* BREWERY YEAST STRAIN CAPACITY OF BINDING WITH MAGNESIUM IN DYNAMIC CONDITIONS

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## ABSTRACT

In this research, the No. 1 *Sacharomyces cerevisiae* brewery yeast strain capacity to bind the $Mg^{2+}$ ions was studied. The yeast were cultivated in dynamic conditions in the YPD medium enriched with the $MgSO_4 \cdot 7H_2O$ or $MgCl_2 \cdot 6H_2O$ magnesium salts. The salts were being added in such an amount, to make the sheer element content in the medium amounting to 0.25 g $\cdot$ $dm^{-3}$; 0.5 g $\cdot$ $dm^{-3}$ or 1.25 g $\cdot$ $dm^{-3}$. The YPD medium was enriched with magnesium ions at the beginning of the cultivation or in the end of the logarithmic phase of yeast growth. In order to evaluate the durability of bonds of $Mg^{2+}$ ions with brewery yeast cells, the magnesium content was indicated in the centrifuged yeast biomass that had and had not been washed with deionized water. The studied strain proved its capacity of permanent bonds with magnesium ions originating from the experimental media. $MgCl_2 \cdot 6H_2O$ added to the cultivation, favoured the binding of magnesium form the YPD medium by the brewery yeast and allowed obtaining slightly higher biomass output than from experimental media with $MgSO_4 \cdot H_2O$ salt. The largest amount of biomass and magnesium that had bound with it (58.71 $Mg^{2+}/dm^3$ of medium) was obtained after 48-hour cultivation with chloric salt added in the amount of 1.25 g $Mg^{2+} \cdot dm^{-3}$ of medium.

**Key words:** bio-elements; metal proteins; bioplex; magnesium; *Saccharomyces cerevisiae*

## INTRODUCTION

Throughout recent years one may observe a certain increase of interest in metallic proteins also known as bioplex. They comprise also proteins binding important metals like zinc, chrome, cobalt, magnesium, selenium and iron. One of the means of binding the proteins with bioelements is the process of binding of microelements with cellular structures of micro-organisms.

Magnesium is a bioelement of a major importance for functioning of living organisms. This bioelement is characteristic for its multidirectional biological activity in a number of metabolic tracks of environment. It acts as an activator of numerous enzymes (mainly kinesis), it participates in the synthesis of proteins, nucleic acids, lipids and thermoregulation [12, 17, 26]. It also positively influences the functioning of muscles and nerves apart from taking part in the regulation of heart muscle functioning and stimulates the development of osseous tissue [18,11]. Recommended daily intake of magnesium is estimated between 300 – 370 mg [7], with the fact that this microelement is exploited by the human body only in 40%, justifies the attempts to manufacture food enriched with this bioelement.

The *Saccharomyces* yeast strain is most frequently used for the research on binding of bioelements by the microorganisms, due to their facilitated supply, easy cultivation and the possibility of obtaining high cellular biomass yield in a relatively short time.

Yeast cells bind double valence metals on their outer surface owing to phosphomannan content in their cell walls and the presence of free carboxyl, hydroxyl, amine, phosphate and hydrosulphide groups in surface proteins [26, 3, 10]. The presence of magesium ions in the environment stimulates their development and prolongs the life of yeast cells. This microelement protects yeast cells from the stress evoked by a high concentration of ethanol, high temperature and osmotic pressure. Small amounts of ethanol, which appear in the preliminary phase of the fermentation process, stimulate the absorption of magnesium in yeast cells, which causes an increase of their activity. Higher ethanol concentrations, being the output of further stages of fermentation, inhibit the development of cells and they are toxic [27]. The highest biosorption of magnesium ions from the medium takes place usually in the first stages of biomass cultivation after which a slower binding of microelement with intracellular structures takes place [5]. In the further stages of cultivation, yeast cells may exhaust those ions into environment together with advancing ageing and cell atrophy process [26].

Optimal magnesium concentration in the medium for the development growth of *Saccharomyces cerevisiae* is between 50 and 100 mg $\cdot$ $dm^{-3}$ whereas the total growth inhibition takes place with 25 g $Mg^{2+} \cdot dm^{-3}$ [13]. The use of yeast cellular biomass enriched with magnesium (or any other bioelements) might possibly constitute means of a natural supplementation of human and animal diet with bioplex, which could have highly beneficial health consequences. Some authors [14, 17] indicate that magnesium absorption by the organism from pharmaceuticals elaborated on the basis of nonorganic or organic salts does not bring a desired level, conversely to magnesium applied in the form of bioplex. The presence of highly absorbable protein, egzogenic aminoacids, the immunal system stimulating and strengthening capacities, as well as probiotical properties of brewery yeast preparations for the herbivore, are the factors that would speak in favour of *Saccharomyces cerevisiae* brewery yeast biomass application for the means of possible diet enrichment with magnesium [1, 2].

The aim of this study was to evaluate the possibility of natural binding of magnesium ions by the *Saccharomyces cerevisiae* brewery yeast cells depending on the type and the amount of added salt in the dynamic conditions. The goal was to obtain a high yield of yeast cellular biomass with possibly the highest amount of permanently bound magnesium. The scope of the study was as follows:

- Assessment of the influence of magnesium salt depending on its type and concentration in the medium on the *Saccharomyces cerevisiae* brewery yeast biomass yield;
- Assessment of magnesium ions binding capacities through *Saccharomyces cerevisiae* yeast cells depending on the type and dose of salt added, and assessment of the most beneficial moment for the introduction of salts into the cultivation medium with respect of the conditions of the experiment.

## MATERIALS AND METHODS

### Biological material

*Saccharomyces cerevisiae* brewery yeast strain No. 1 originating from the collection of Pure Cultures Biotechnology and Agricultural and Food Industry Institute in Warsaw was used in the study. The yeast was stored on the malt slants in the temperature of 4°C and every 4 weeks transferred on the fresh medium.

*Inoculum* was prepared by injection of the YPD medium with the material originating from the slants. The cultivation period was 24 hours in the temperature of 28°C with the inoculum placed upon the shaker ROSI 1000 (Thermolyne, USA) at 200 rpm. Obtained inoculum constituted a base material for the given series of tests.

### Microbiological media

**The malt extract with agar** [8] was used to store the *Saccharomyces cereviasiae* yeast.

**YPD medium with 2% agar** [23, 4] was used to count the yeast cells with the use of plate method which aimed at establishing the growth curve of the yeast strain.

YPD medium was used **as a control medium** for dynamic batch cultivation.

**As an experimental media**, the YPD medium enriched with magnesium ions originating from two compounds: $MgCl_2 \cdot 6H_2O$ or $MgSO_4 \cdot 7H_2O$ was used. Three levels of magnesium salt addition were accepted so that the magnesium content in the medium was 0.25 g $\cdot$ $dm^{-3}$; 0.5 g $\cdot$ $dm^{-3}$ and 1.25 g $\cdot$ $dm^{-3}$.

### Yeast cultivation in control and experimental media enriched with magnesium ions

To enrich the *Sacharomyces cerevisiae* brewery yeast cells with magnesium, two variants of cultivation were applied in liquid media:

Variant I: Magnesium ions were added to the YPD medium as $MgCl_2 \cdot 6H_2O$ or $MgSO_4 \cdot 7H_2O$ in the beginning of the cultivation. This variant was labelled as t = 0.

Variant II: Magnesium ions (as $MgCl_2 \cdot 6H_2O$ or $MgSO_4 \cdot 7H_2O$) were added to the YPD medium in the end of the logarithmic phase of yeast growth. This variant was labelled t = 24.

Yeast was cultivated in experimental & control media in dynamic process with the application of shaker (Buhler SM-30 Control, Germany) in the temperature of 28°C during 48 hours. At least three series of tests were held, each including every variant of cultivation. During the experiment the yield of yeast biomass and the content of magnesium in the cellular biomass were controlled. The samples were evaluated in the 0, 24th and 48th hour of cultivation for the variant I and in 24th and 48th hour of cultivation for the variant II. The reason for this is that at the beginning of the cultivation in the variant II the biomass yield and the content of magnesium in the biomass are the same as in the cultivation in the control medium. In the variant II salt of magnesium has been added to the YPD media not earlier than 24 hours from the beginning of the cultivation process, that is in the end of the logarithmic growth phase.

### Analytical part

### Evaluation of yeast cellular biomass yield

The yeast cellular biomass yield was evaluated by centrifuging 8 $cm^3$ of the experimental (or control) medium for 10 minutes at 3500 rpm (MPW-365 centrifuge, Poland). The liquid above the residue level was poured out and the cellular biomass was dried firstly in the temp 60°C for 2 hours, then it was additionally dried in the temp. 105°C to dry weight. The results were given in g of d.w. $\cdot$ $dm^{-3}$ of the medium.

### Evaluation of magnesium content in the yeast cellular biomass with the use of ASA

The content of magnesium in the yeast cellular biomass obtained from the control and experimental media was evaluated after 0, 24, and 48 hours of cultivation. Centrifuged, dried and weighted yeast biomass originating from particular cultivation was mineralised in the mixture of nitric and perchlorate acids. The magnesium

content was evaluated in the samples prepared in this way with the use of ASA method (spectrophotometer Schimazu AA660, Japan) [6]. The absorbance was measured with the length of waves 285.2 nm. The results were specified in the mg $Mg^{2+}$/ g. s.s. of yeast biomass.

**Statistical analysis**

Obtained results were subjected to statistical analysis, which was conducted with the use of statistical packet Statgraphics Plus.

The relevance of the influence of different doses of magnesium salts being the content of the media on the biomass yield and the magnesium content in the yeast cellular biomass was studied. The variance analysis was conducted with the use of Tukey test for the level of alpha relevance = 0.05. The results of statistical analysis are presented in Tables 1-7.

**Stages of research, presentation of results and discussion**

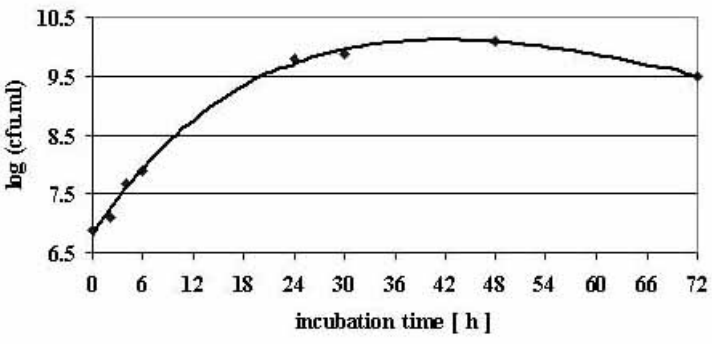
**The growth curve determination for *S. cerevisiae* brewery yeast**

The main goal of this study was to obtain the highest amount of yeast cellular biomass with a high amount of permanently bound magnesium. It is obvious that magnesium salts being added in different concentrations to the medium have a stimulating or inhibitory influence upon the amount of obtained cellular biomass. That is the reason why parallely to the series where magnesium was added to the YPD experimental medium in the beginning of the cultivation (variant I, t = 0), a series of tests was conducted in which the cultivatived medium was enriched with magnesium ions but only where the amount of yeast cells was evaluated to be the highest. Theoretically it is known that such a moment takes place at the end of the logarithmic growth phase.

In order to determine the end of the logarithmic growth phase, a growth curve in the conditions of dynamic cultivation was established with the use of plate count method.

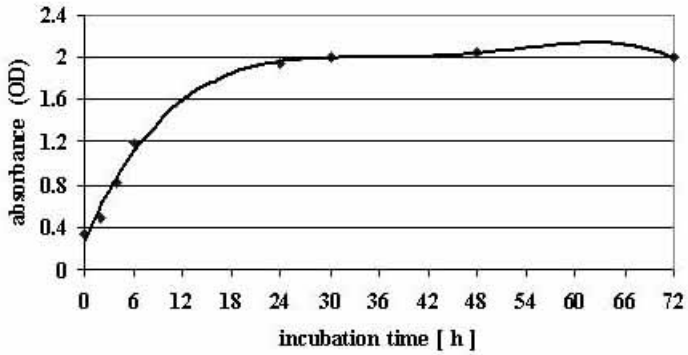
In Figure 1, the dependence of yeast cell logarithm on the time of cultivation was presented. The highest amount of yeast cells in the YPD medium was indicated in the 48th hour of cultivation. The most dynamic increase in the number of yeast cells was observed in the period before the 24th hour of cultivation, that is why in the subsequent part of research, that moment was accepted as the final point of the logarithmic growth phase and it was claimed to be the best moment for the introduction of magnesium ions into the YPD medium (variant II, t = 24). Changes in absorbance (optical density) during the *S. cerevisiae* growth period in the YPD medium and in the conditions of dynamic cultivation were observed in order to make sure that in the further tests the 10% (v/v) 24 hour inoculum in the control and experimental media guarantees introduction of possibly the same amount of yeast cells.

**Fig. 1. Growth of *S. cerevisiae* on YPD medium during cultivation in dynamic conditions**




In Figure 2 the dependence of optical density (wave length = 600 nm) of YPD medium during the growth period of *S. cerevisiae* brewery yeast upon the duration of cultivation was presented.

**Fig. 2. Changes in absorbance (v.l. = 600 nm) on YPD medium during cultivation of S. cerevisiae *in dynamic conditions***




Optical density was increasing at a high rate before the 24th hour of cultivation (logarithmic increase in the number of yeast cells) to stabilise at the level of about 2.0 which remained stable for the rest of the observation period.

Control and experimental media in particular series of research were inoculated when the optical density of inoculum attained the level of about 2.0.

**The influence of magnesium ions upon the *S. cerevisiae* cellular biomass yield**

At this stage of research the aim was to evaluate which of the magnesium salts added to the experimental media influenced the amount of yeast cellular biomass yield. A statistical analysis was conducted, stating whether the doses of magnesium that had been applied influenced considerably the biomass growth.

The results obtained in the I and II variant of cultivation, were presented in the Tables 1-3.

**Table 1. Yield of *S. cerevisiae* biomass during the cultivation in the control and experimental mediums enriched with $MgCl_2 \cdot 6H_2O$ (variant I, t = 0)**

| Medium type | Cultivation time [h] Yield of biomass [g of d. w. · $dm^{-3}$] | | |
|---|---|---|---|
| | 0 | 24 | 48 |
| YPD (control) | 1.17a | 7.89b | 10.33b |
| YPD + 0.25 g $Mg^{2+} \cdot dm^{-3}$ | 1.32a | 8.52b | 10.85b |
| YPD + 0.50 g $Mg^{2+} \cdot dm^{-3}$ | 1.28a | 8.57b | 11.00b |
| YPD + 1.25 g $Mg^{2+} \cdot dm^{-3}$ | 1.21a | 8.94b | 11.20b |

**means with the same letter did not differ significantly**

**Table 2. Yield of *S. cerevisiae* biomass during the cultivation in the control and experimental mediums enriched with $MgSO_4 \cdot 7H_2O$ (variant I, t = 0)**

| Medium type | Cultivation time [h] Yield of biomass [g of d. w. · $dm^{-3}$] | | |
|---|---|---|---|
| | 0 | 24 | 48 |
| YPD (control) | 1.21a | 8.54b | 10.60b |
| YPD + 0.25 g $Mg^{2+} \cdot dm^{-3}$ | 1.30a | 8.46b | 10.63b |
| YPD + 0.50 g $Mg^{2+} \cdot dm^{-3}$ | 1.34a | 8.70b | 10.64b |
| YPD + 1.25 g $Mg^{2+} \cdot dm^{-3}$ | 1.21a | 8.43b | 11.15b |

**means with the same letter did not differ significantly**

**Table 3. Yield of *S. cerevisiae* biomass during the cultivation in the control and experimental mediums enriched with $MgCl_2 \cdot 6H_2O$ (variant II, t = 24)**

| Medium type | Cultivation time [h] Yield of biomass [g of d. w. · $dm^{-3}$] | |
|---|---|---|
| | 24 | 48 |
| YPD (control) | 7.40a | 10.58b |
| YPD + 0.25 g $Mg^{2+} \cdot dm^{-3}$ | 7.68a | 10.73b |
| YPD + 0.50 g $Mg^{2+} \cdot dm^{-3}$ | 7.23a | 10.60b |
| YPD + 1.25 g $Mg^{2+} \cdot dm^{-3}$ | 7.59a | 10.72b |

**means with the same letter did not differ significantly**

It was stated that yeast being cultivated in the experimental media (enriched with magnesium ions being the $MgCl_2$ or $MgSO_4$ salts) indicated usually slightly higher biomass yield than the yeast cultivated in the control media (with no $Mg^{2+}$ ions added), nevertheless the differences observed in the studied time periods were not significant.

For both sources of magnesium salts, a higher biomass yield was obtained after 48 hours of cultivation.

The highest cellular biomass yield was observed after 48 hours of cultivation in the experimental medium containing 1.25 g of $Mg^{2+} \cdot dm^{-3}$ (variant I, t = 0). It amounted to 11.20 g.d.w · $dm^{-3}$ of medium for $MgCl_2$ salt and 11.15 g.d.w. · $dm^{-3}$ of medium for $MgSO_4$ salt, respectively.

Due to the lack of any significant differences and slightly higher biomass yield from the media containing 0.25 g and 0.50 g of magnesium ions $Mg^{2+} \cdot dm^{-3}$ originating from $MgCl_2$, it has been decided to concentrate only on this kind of salt in the further research.

During the yeast cultivation in the YPD medium with $MgCl_2$ being introduced after 24 hours (variant II, t = 24) that is after the logarithmic growth phase, no significant differences in the cellular biomass yield in the experimental and control medium were observed (Table 3).

Comparing the biomass yield obtained in variant II of the experiment with the one where magnesium was added into the medium at the beginning of the cultivation (Table 1), it was stated that a slightly larger amount of biomass was obtained in the variant I.

The variety of salt and final concentration of magnesium ions in the YPD media in the conditions of the experiment had no significant influence on the *S. cerevisiae* yeast cellular biomass yield. It should also be stated that magnesium ions present in the environment did not have any inhibitory effects on the growth of the strain being tested. Similar results were obtained by Pasternakiewicz and Tuszyński [24], who indicated that adding magnesium to the cultivative wort in the amount of 0.1-1.2 g of $Mg^{2+} \cdot dm^{-3}$ of medium did not cause a decrease of biomass yield as compared to the yeast cultivated in the wort not enriched with this element.

It is also comparable with the results presented by other authors [20, 21, 22] who state that toxic dose of magnesium for yeast is high and amounts to about 25 g of $Mg^{2+} \cdot dm^{-3}$ of medium. Concentrations of magnesium ions used in this study fitted into the range optimal for the *S. cerevisiae* growth [13] which created a chance of obtaining a more permanent magnesium bound with yeast cells together with sustaining high biomass yield.

**Magnesium binding capacities of *S. cerevisiae* yeast cells from control and experimental media**

Magnesium content in the cellular biomass of the yeast cultivated with the use of dynamic method in the control medium and experimental media was evaluated in variant I directly after their application in the media used and after 24 and 48 hours of cultivation. The tests aimed a checking magnesium binding capacities of a given yeast strain depending on the ion dose and the type of magnesium salt being introduced into the experimental medium. To assess the durability of the bound between this element and yeast cellular structures, there have been parallel tests conducted, where the biomass was washed two times with deionized water before the magnesium content was evaluated. Water, having a dipolar character, was to eliminate those parts of magnesium ions that had not penetrated the cell by the means of active transport but had only attached loosely to the intracellular spaces of yeast biomass.

Obtained results, having been statistically elaborated, were presented in Tables 4-6. According to the results, the differences between magnesium content in biomass before and after washing it with deionized water, are very big. It speaks in favour of adopting a hypothesis that only a part of magnesium is actively transported into the inside of cells to be bound there with their structures, while the remaining $Mg^{2+}$ ions are absorbed only on the outer layer of yeast cellular wall.

**Table 4. Yield of *S. cerevisiae* biomass during the cultivation in the control and experimental mediums enriched with $MgCl_2 \cdot 6H_2O$ (variant I, t = 0)**

| Medium type | Cultivation time [h] Yield of biomass [g of d. w. · $dm^{-3}$] | | | | | |
|---|---|---|---|---|---|---|
| | Biomass without washing | | | Biomass with washing | | |
| | 0 | 24 | 48 | 0 | 24 | 48 |
| YPD (control) | 2.22a | 1.79a | 1.53a | 2.22A | 1.79A | 1.53A |
| YPD + 0.25 g $Mg^{2+} \cdot dm^{-3}$ | 5.81a | 3.34a | 3.54a | 5.78B | 3.02A | 2.77A |
| YPD + 0.50 g $Mg^{2+} \cdot dm^{-3}$ | 10.01b | 4.62ab | 5.84ab | 5.74B | 3.03A | 3.31A |
| YPD + 1.25 g $Mg^{2+} \cdot dm^{-3}$ | 18.11c | 8.11ab | 8.43ab | 6.71B | 4.02AB | 5.77AB |

**means with the same letter did not differ significantly**

**Table 5. Yield of *S. cerevisiae* biomass during the cultivation in the control and experimental mediums enriched with $MgSO_4 \cdot 7H_2O$ (variant I, t = 0)**

| Medium type | Cultivation time [h] Yield of biomass [g of d. w. · $dm^{-3}$] | | | | | |
|---|---|---|---|---|---|---|
| | Biomass without washing | | | Biomass with washing | | |
| | 0 | 24 | 48 | 0 | 24 | 48 |
| YPD (control) | 2.08a | 1.67a | 1.89a | 2.08A | 1.67A | 1.89A |
| YPD + 0.25 g $Mg^{2+} \cdot dm^{-3}$ | 5.35a | 2.41a | 3.45a | 4.00B | 2.33A | 2.07A |
| YPD + 0.50 g $Mg^{2+} \cdot dm^{-3}$ | 7.25ab | 3.31a | 4.57a | 4.07B | 2.35A | 2.76A |
| YPD + 1.25 g $Mg^{2+} \cdot dm^{-3}$ | 17.11b | 8.19ab | 8.32ab | 4.02B | 3.91B | 5.01B |

**means with the same letter did not differ significantly**

**Table 6. Yield of *S. cerevisiae* biomass during the cultivation in the control and experimental mediums enriched with $MgCl_2 \cdot 6H_2O$ (variant II, t = 24)**

| Medium type | Cultivation time [h] Yield of biomass [g of d. w. · $dm^{-3}$] | | | |
|---|---|---|---|---|
| | Biomass without washing | | Biomass with washing | |
| | 24 | 48 | 24 | 48 |
| YPD (control) | 1.88a | 1.59a | 1.88A | 1.59A |
| YPD + 0.25 g $Mg^{2+} \cdot dm^{-3}$ | 2.80b | 3.16b | 2.09A | 2.76B |
| YPD + 0.50 g $Mg^{2+} \cdot dm^{-3}$ | 3.98ab | 4.11ab | 2.68B | 3.44B |
| YPD + 1.25 g $Mg^{2+} \cdot dm^{-3}$ | 6.04bc | 7.07c | 3.15B | 4.24AB |

**means with the same letter did not differ significantly**

The more magnesium was in the experimental medium, the larger amount of it was loosely bound with the cellular wall (e.g. in the biomass, originating from the experimental medium, which was not washed with an addition of 1.25 g of $Mg^{2+} \cdot dm^{-3}$, magnesium content was 8 times larger than in the control medium and amounted to 18.11 and 2.22 mg of $Mg^{2+}$/g.d.w., respectively). Nonetheless, the bond was so weak that after a double wash with deionized water and centrifugation, magnesium was detaching from the cells and entering the medium in the amount exceeding even 60% of its initial content (Tables 4-5).

The aim of this study was to obtain yeast that would bind magnesium permanently, that is why most attention was devoted to magnesium content in yeast cells washed with deionized water (after centrifugation of biomass originating from cultivation in control and experimental media).

The results dealing with magnesium content in yeast that had not been washed, were treated as auxiliary data, as they related to magnesium loosely bound with the *S. cerevisiae* cellular structures.

In both variants of dynamic cultivation (Tables 4-6) it was observed that usually the highest amount of magnesium accumulates in the biomass during the initial hours of cultivation. It might mean that yeast in the logarithmic growth phase is characterised by high demand of magnesium, whereas in the stages of retarded growth and stationary cell, it liberates a part of this element into the medium. Fast magnesium absorption in the logarithmic growth phase is caused by the vitality of cells that are budding intensively. At this stage, the presence of $Mg^{2+}$ ions [25, 26, 3] in the environment is necessary for the multiplying yeast. This element participates in the synthesis of the DNA [9] activating polimerase of the DNA being the enzyme responsible for replication. As it is stated by Mowll and Gadd [16], in the initial stage of cultivation, an intense magnesium binding with yeast cell wall structures takes place (the first stage of magnesium accumulation). Bio-accumulation is the second stage; it could be described as a slower but more permanent binding of these ions with the yeast intracellular structures. From the point of view of the aims of this study, the second stage of magnesium accumulation seems more interesting. It is yet not clear at which point of cultivation did magnesium enter into permanent binding with yeast cells.

On the basis of the conducted research, a significant influence of magnesium salts applied in the experimental media on its binding with cellular biomass was indicated. In the dynamic method ($MgCl_2$ as the source) the largest amount of permanently bound magnesium (6.71 mg of $Mg^{2+}$/g.d.w.) was obtained in the variant I of cultivation (directly after the inoculation of experimental medium) with the dose of 1.25 g/dm$^3$ (Table 4). This result was three times higher in relation to control test.

In the dynamic method ($MgSO_4$ as the source), the largest amount of magnesium (5.01 mg of $Mg^{2+}$/g.d.w.) was obtained for the dose of 1.25 g $\cdot$ dm$^{-3}$ at the 48th hour of cultivation which was over 2,5 times more magnesium than in the control test (Table 5).

According to Tuszyński and Pasternakiewicz [24], magnesium content in the biomass after 40 hours of dynamic cultivation in the medium containing 20 mM of $Mg^{2+}$ ions (i.e. about 0.5 g $Mg^{2+} \cdot dm^{-3}$) amounted to around 2 mg/g.d.w. In this study with the application of the same dose of magnesium, after 48 hours of cultivation, obtained results were slightly higher.

$MgCl_2$ salt being used as a source of magnesium, 3.13 mg of $Mg^{2+}$/g.d.w. were obtained (Table 4) while 2.76 mg of $Mg^{2+}$/g.d.w. of yeast biomass were obtained with the second salt type-$MgSO_4$ (Table 5). Those differences could be the result of using different sources of magnesium by the authors ($MgNO_3$) or another experimental medium (brewery wort).

In cultivations where chloric salt was the source of magnesium for the yeast, larger amount of magnesium being permanently bound with the cellular biomass was indicated than in the cultivations where magnesium was added under the form of sulphuric salt (even 50% difference for the 1.25 g $Mg^{2+} \cdot dm^{-3}$ dose - Tables 4-5).

That is why in variant II of the cultivation, $MgSO_4$ has been rejected as the source of magnesium (magnesium added into experimental YPD medium in the end of the logarithmic yeast growth phase).

Subsequent cultivations in experimental media were to check whether adding of magnesium in the end of logarithmic growth phase improves the effectiveness of its bound with the yeast cells (Table 6). In this variant of experiment the highest amount of magnesium was found in the yeast cellular biomass after 48 hours of cultivation (4.24 mg of $Mg^{2+}$ /g.d.w. were obtained in the medium containing the highest dose of $Mg^{2+}$). The application of 0.5 g dose of $Mg^{2+} \cdot dm^{-3}$ and 1.25 g of $Mg^{2+} \cdot dm^{-3}$ had a significant influence on the magnesium content in the biomass in comparison to the control test. Cultivations where magnesium was added to the media in the end of the logarithmic growth phase (t = 24) did not prove to contain larger amounts of this element in the yeast biomass as compared to analogous cultivations in variant I (t = 0).

Obtained results prove that magnesium is necessary for the yeast to grow, especially at initial hours of cultivation. After the stage of logarithmic growth phase, a part of magnesium may be liberated from the cells into the medium probably due to the undergoing processes or certain defensive mechanisms of the yeast aiming at protecting it from over-saturation with magnesium. It seems that in the conditions of the experiment (i.e. the cultivation in YPD medium) maximal possible level of *S. cerevisiae* brewery yeast biomass saturation with magnesium was about 6 mg of $Mg^{2+}$/ g d.w.

The amount of magnesium being not permanently bound with the cells rose with the increase of $Mg^{2+}$ ions being added into experimental media (Tables 4-5). Such tendency was not observed in the case of magnesium content permanently being bound with cellular biomass. The amount of magnesium was independent of the scope of $Mg^{2+}$ ions in the experimental medium (Table 4). It may be assumed that the YPD medium, rich in substantial elements being optimal for the yeast growth [15], established for the tested yeast brewery a certain boarder of magnesium availability, above which well nourished cells will not naturally bind any more $Mg^{2+}$ ions.

As it has already been stated, obtaining the highest possible yield of brewery yeast cellular biomass with permanently bound magnesium ions was the aim of the experiment. In order to improve the binding, the two distinguished elements were presented as a result of yield multiplying (of the biomass obtained in the variant I for the chloric salt) with the magnesium content in the yeast dry mass (Table 7). Similarly to earlier results, only those that originated from the cultivations obtained after washing the yeast with deionized water, were subjected to interpretation.

**Table 7. Yield of *S. cerevisiae* biomass during the cultivation in the control and experimental mediums enriched with $MgCl_2 \cdot 6H_2O$ (variant I, t = 0)**

| Medium type | Cultivation time [h] Yield of biomass [g of d. w. · $dm^{-3}$] | | | | | |
|---|---|---|---|---|---|---|
| | Biomass without washing | | | Biomass with washing | | |
| | 0 | 24 | 48 | 0 | 24 | 48 |
| YPD (control) | 2.61a | 14.10a | 15.75a | 2.61A | 14.10A | 15.75A |
| YPD + 0.25 g $Mg^{2+} \cdot dm^{-3}$ | 7.65a | 28.47a | 38.41ab | 4.38A | 23.28B | 28.27B |
| YPD + 0.50 g $Mg^{2+} \cdot dm^{-3}$ | 12.81a | 39.60ab | 64.25b | 4.57A | 23.89B | 31.47B |
| YPD + 1.25 g $Mg^{2+} \cdot dm^{-3}$ | 21.91a | 72.53b | 94.44bc | 5.25A | 31.79B | 58.71B |

**means with the same letter did not differ significantly**

The best result, i.e. 58.71 mg of $Mg^{2+} \cdot dm^{-3}$ of medium was indicated after 48-hour cultivation (variant I, t=0) with magnesium being added under the from of $MgCl_2$ in the amount of 1.25 g · $dm^{-3}$.

The results suggest that magnesium determined after washing the biomass with deionized water was permanently bound with yeast cells (cellular wall or intracellular structures under the form of bioplex).

Localisation of magnesium in brewery yeast cells will be the subject of further research conducted by the authors of this study.

## CONCLUSIONS

The conclusions drawn on the results of the study are as follows:

1. *S. cerevisiae* strain No 1 that was applied the study proved to have the capacity of permanent binding of $Mg^{2+}$ ions from experimental media (being a content of sulphuric or chloric salt of this element) in the tested scope of concentrations.
2. Enrichment of YPD media with magnesium ions at the concentrations of 0.25, 0.5 and 1.25 g $Mg^{2+} \cdot dm^{-3}$ did not cause any significant increase in cellular biomass yield in the conditions of the experiment when compared to the YPD control medium (with no magnesium added).
3. Large amounts of magnesium in the conditions of the experiment (even over 60% of initial content in the biomass) were bound temporarily with the yeast cellular wall. It was indicated as a result of cellular biomass being washed with deionized water. Magnesium, which remained in the yeast has probably bound permanently with their cellular structures.
4. The biomass of studied brewery yeast obtained from the cultivation in the experimental media enriched with $Mg^{2+}$ ions usually contained 2-3 times more magnesium permanently bound with the cells than from the cultivation in the control medium.
5. Logarithmic growth phase helped the *S. cerevisiae* brewery yeast cells to bind $Mg^{2+}$ ions from the medium.
6. The largest amount of brewery yeast cellular biomass and magnesium being permanently bound with it (58.71 mg $Mg^{2+} \cdot dm^{-3}$ of medium) was obtained as a result of a 48-hour dynamic cultivation in the YPD medium with $MgCl_2$ salt added in the amount of 1.25g $Mg^{2+} \cdot dm^{-3}$ introduced at the stage of logarithmic cell growth.

## ACKNOWLEDGMENT

This work was financially supported by the KBN grant No. 6 P06T 065 21.

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