COPPER SUPPLEMENTED YEAST BIOMASS AS A SOURCE OF NUTRITION MICROELEMENTS

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ABSTRACT

Copper is very important microelement found in all organs and tissues of the human body. This study discusses the possibility of intake of this microelement in natural form; therefore the biomass of yeast can be used. Our research aim was to investigate the influence of copper on the yield of yeast biomass and to determine the suitability of *Saccharomyces cerevisiae* strain 612 to absorb the copper from the medium to the yeast cells. Three forms, i.e. copper nitrate, copper sulphate and copper chloride each one in six different concentrations were used. Metal concentration in the yeast biomass was determined using AAS method. The highest increase of copper in yeast biomass, six times, i.e. 21.6 mg.kg^{-1}, was recorded after 48 hours of submersed cultivation on YPD medium with addition of 100 µg.100 mL^{-1} in the form of copper sulphate.

Keywords: copper, yeast, yield, biomass, *Saccharomyces cerevisiae*

INTRODUCTION

The role of biogenic microelements, i.e. zinc, copper, magnesium, selenium, iron in the metabolic state of microorganisms and higher organisms (humans and animals) has become an especially interesting field for research. Microelements play an important role in the cellular metabolism, primarily as cofactor for a large number of enzymes involved in a variety of biological reactions, such as respiration, free radical eradication, connective tissue formation, iron metabolism and neurological function. The number of studies of the processes involved in the uptake of trace elements by the yeast *Saccharomyces cerevisiae* has increased considerably in recent years. This yeast has become a model microorganism for studying metal transporters and their accumulation in the yeast cells. All living organisms require copper for growth and development **(Magálová *et al.*, 1997; Mapolelo, Torto, 2004; Stehlik-Tomas *et al.*, 2004; Kuo *et al.*, 2006)**.

Copper is actively absorbed from the blood in the upper small intestine. In this process play an important role amino acids and specific protein, which contains sulphydryl groups. After an absorption in gastro-intestinal tract, copper is bound to albumin and is transported to the liver **(Skalická *et al.*, 2009)**.

The adult human body contains about 70 – 100 mg of copper. The highest concentration of copper ions (in decreasing order) occurs in liver, brain, heart and kidney. Muscles contain about 50 % of total body copper. Human erythrocytes contain 1.0 – 1.4 µg.mL^{-1} of copper, of which more than 60 % is in superoxide dismutase **(Bhagavan, 2002; Prohaska, Gybina, 2004)**.

Many enzymes, i.e. ceruloplasmin, superoxide dismutase, cytochrome c oxidase and tyrosinase require copper for their activity. Ceruloplasmin catalyses the conversion of ferric ions to the ferrous complex, favoring the absorption of iron from the gastro–intestinal tract. It also plays a role in the mobilization of iron to plasma from the tissue **(Pathak, Kapil, 2004)**. Copper is necessary for bone formation, energy metabolism, nerve transmission, elastin synthesis, pigmentation of the skin, normal hair growth and red blood cell production **(Pang *et al.*, 2001)**.

MATERIAL AND METHODOLOGY

Saccharomyces cerevisiae Meyen ex E.C. Hansen strain 612 was obtained from the distillery Slovenské liehovary a likérky, a. s. Leopoldov, Slovakia. The yeast was routinely maintained on Malt Extract agar for microbiology (Merck, Germany) and was grown on Yeast Peptone Dextrose medium (YPD) **(Šillerová *et al.*, 2010)**.

Based on previous literature sources **(Stehlik-Tomas *et al.*, 2004; Wang, Chen, 2006; Barbulescu *et al.*, 2010)** the stock solutions were prepared in concentration of 0.01 % w/v of all three copper forms, i.e. $Cu(NO_3)_2 . 3 H_2O$ (POCH, Poland), $CuSO_4 . 5 H_2O$ and $CuCl_2 . 2 H_2O$ (Lachema, Czech Republic). The solutions were prepared in non-H_2O form in deionised water with natural pH of 5.4 – 5.6.

Copper was added from the stock solutions to the final concentrations of 20, 40, 60, 80, 100 µg.100 mL^{-1}, respectively. YPD medium with required concentration of copper was inoculated with sterile loop and cultivated by submersed cultivation under aerobic conditions on an orbital shaker (MEZ, Czechoslovakia; 280 rpm) at 30 °C in dark for 48 h. Biomass was harvested from liquid medium by centrifugation (K 70 D, Engelsdorf/Leipzig; 1509 x g, 30 min), and rinsed with distilled water. Obtained material was lyophilized (LYOVAC GT 2, AMSCO/FINN-AQUA, Germany) in order to observe yeast biomass without losing its biological activity.

Copper cell content in yeast biomass was analysed by atomic absorption spectrophotometer method (AAS; Varian FS240). The software STATISTICA (StatSoft, Inc. (2005). STATISTICA Cz, version 7.1.), one-way analysis of variance (ANOVA) for data analysis was used. Results for cellular copper content in yeast and the influence of copper on the yield of biomass were performed with the Fisher's LSD test at $P = 0.05$. Data are presented as means ± SD.

RESULTS AND DISCUSION

The influence of copper nitrate, copper sulphate and copper chloride on the yield of yeast biomass was determined as shown in Tables 1 – 3.

Table 1 Influence of copper nitrate on the yield of yeast biomass

Concentration of Cu, µg	Average yield of yeast biomass, g.100 mL^{-1} YPD
0	$1.724_a \pm 0.116$
20	$1.735_a \pm 0.257$
40	$1.718_a \pm 0.312$
60	$1.841_a \pm 1.064$
80	$1.825_a \pm 0.681$
100	$1.646_a \pm 0.175$

Values are means ± SD, n = 9.

Based on results it was shown that no differences were significant (P_1 = 0.985). It means that all concentrations of copper nitrate didn't cause decreasing or increasing yield of yeast biomass (Table 1). It was obtained 1.7 g of dry yeast biomass from 100 ml YPD.

Table 2 Influence of copper sulphate on the yield of yeast biomass

Concentration of Cu, µg	Average yield of yeast biomass, g.100 mL^{-1} YPD
0	$1.724_a \pm 0.116$
20	$1.764_a \pm 0.095$
40	$1.672_a \pm 0.169$
60	$1.782_a \pm 0.343$
80	$1.773_a \pm 0.572$
100	$1.760_a \pm 0.864$

Values are means ± SD, n = 9.

According to the added concentrations of copper sulphate (Table 2) and copper chloride (Table 3) there are no significant differences in the yield of biomass, P_2 = 0.998, P_3 = 0.999, respectively. The changes in the obtained amount of dry yeast biomass were inconsiderable.

Table 3 Influence of copper chloride on the yield of yeast biomass

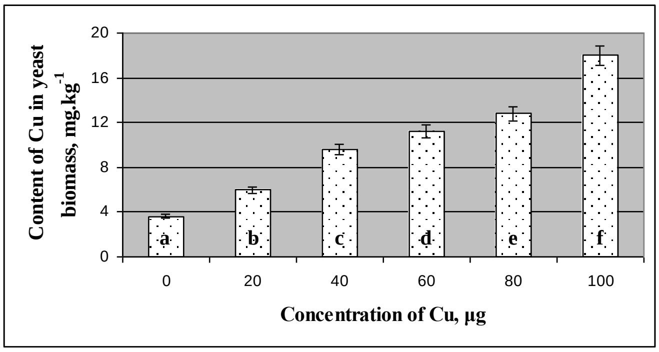
Concentration of Cu, µg	Average yield of yeast biomass, g.100 mL^{-1} YPD
0	$1.724_a \pm 0.116$
20	$1.779_a \pm 0.584$
40	$1.778_a \pm 0.421$
60	$1.782_a \pm 0.786$
80	$1.778_a \pm 0.841$
100	$1.794_a \pm 0.399$

Values are means ± SD, n = 9.

Silóniz *et al*. (2002) reported that higher concentrations of copper affected both morphology and physiological parameters of the viable yeast, and it is thought that a process of bioaccumulation may be involved in its copper uptake. The growth rate of cells decreased with increasing concentrations of copper, mainly due to a decrease in the biomass yield. Higher copper concentrations markedly decreased yeast cell growth rate **(Mrvčić *et al*., 2007)**.

The potentially changing content of copper in the yeast biomass according to the added forms of copper was studied (Figures 1 – 3).

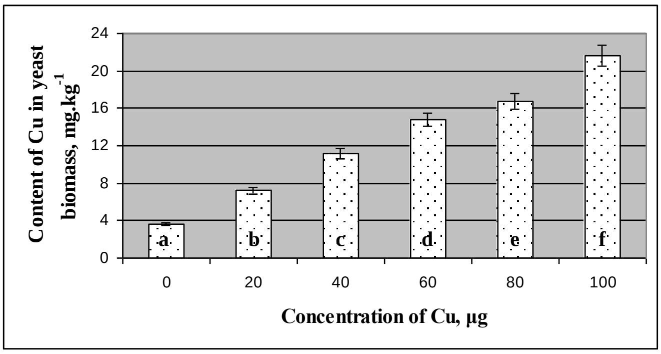
Figure 1 Influence of added Cu concentrations in the form of copper nitrate on the content of Cu in yeast biomass



Values are means ± SD, n = 6.

The yeast biomass without addition of copper contained 3.6 mg.kg^{-1} Cu. Using higher concentrations of copper, the content of Cu was progressively increased (Figure 1). The yeast biomass cultivated in YPD medium enriched with 100 µg of copper nitrate accumulated 5 times more copper than when cultivated in the medium without addition of this metal.

Figure 2 Influence of added Cu concentrations in the form of copper sulphate on the content of Cu in yeast biomass

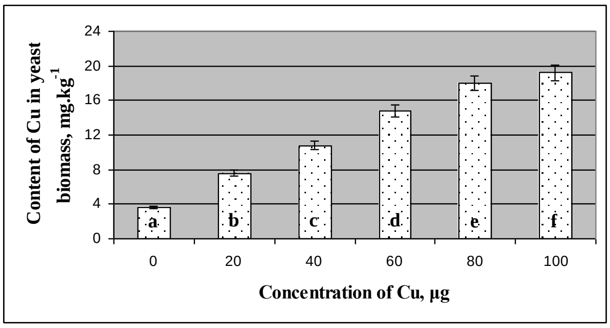


Values are means ± SD, n = 6.

The applied concentrations of copper sulphate significantly influenced the content of copper in yeast cells (Figure 2). The highest value of Cu, i.e. 21.6 mg.kg^{-1} of dry yeast biomass, which represent 6 times increasing of copper content in yeast cells was reached using 100 µg of copper sulphate in the medium.

The effect of copper chloride on the changing of copper content in the yeast biomass was studied (Figure 3). It was found that using the lowest tested concentration of copper chloride, i.e. 20 µg, caused more than 2 times higher Cu amount in the yeast cells.

Figure 3 Influence of added Cu concentrations in the form of copper chloride on the content of Cu in yeast biomass



Values are means ± SD, n = 6.

According to the research carried out, it was shown that tested concentrations of all three forms, i.e. copper nitrate, copper sulphate and copper chloride caused significant differences in the content of accumulated copper in the analysed biomass, P = 0.000.

Stehlik-Tomas *et al*. (2004) reported that the highest value of copper ions in dry matter was 1.1 $mg.g^{-1}$. The maximum amount of copper uptake was 1.16 $mg.g^{-1}$ **(Mrvčić *et al*., 2007)**. It was determined that up to 20 mg of copper per gram of viable adapted biomass could be accumulate from the medium **(Silóniz *et al*., 2002)**.

Our results represent lower content of Cu in the yeast biomass because of the lower input of copper into the medium.

This research will continue with addition of increasing concentrations of copper to determinate the level, which is toxic for yeast and cause the death of these microorganisms.

CONCLUSION

This study confirmed the possibility of accumulation of copper by *Saccharomyces cerevisiae*. All used forms of copper did not cause lower or higher yield of yeast biomass. The tested concentrations of copper are significantly important for absorption of this metal. It was shown that the copper content in the yeast biomass depends only on the concentration of copper obtained in the medium. The highest content of copper, i.e. 21.6 $mg.kg^{-1}$ of dry yeast biomass, which represent 6 times increasing of copper content in yeast cells was obtained adding 100 µg of copper sulphate in the medium.

Copper enriched biomass can be potentially used as a supplement of this important microelement in human nutrition.

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Acknowledgments:
This work was supported by projects KEGA 334-013SPU-4/2010 and VEGA 1/0740/08.

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