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ORIGINAL PAPERS

PRODUCTION OF ZINC-ENRICHED BIOMASS OF *SACCHAROMYCES CEREVISIAE*

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Abstract

Zinc accumulation and the growth of *Saccharomyces cerevisiae* were investigated in a culture with zinc sulfate-supplemented medium. The cultivations were performed on Sabouraud dextrose broth medium in aerobic conditions, without the addition of zinc (control culture) and with the addition of zinc sulfate (5, 10, 15, 30 and 60 mg $ZnSO_4$ l^{-1} medium) at 28°C for 72 hours. The results showed similar trends of yeast growth rates at 24, 48, and 72-hour interval, with concentrations above 10 mg l^{-1} $ZnSO_4$ in the nutritional medium significantly decreasing the yeast growth rate and the biomass yield ($P<0.05$). Substantial differences between the initial $ZnSO_4$ concentrations in the growth medium were demonstrated in the overall adsorption of Zn ions (Zn^{2+}) in yeast cells by a colorimetric assay ($P<0.05$). Similarly, the content of total accumulated zinc, as well as the fractions of Zn present in cells depended mainly on the zinc concentration in the medium, as the total Zn accumulation and organically bound Zn fractions were increased by elevating the $ZnSO_4$ supplementation in the culture medium up to 30 mg l^{-1}, but gradually reduced by any further addition of $ZnSO_4$ determined by an ICP-MASS assay ($P<0.05$). In the presence of 30 mg l^{-1} $ZnSO_4$, the Zn content in the biomass increased by 24-fold, to 4132.34 μg g^{-1} in comparison to 171.9 μg g^{-1} achieved in the basal medium. Thus, the ability of *S. cerevisiae* to accumulate zinc can be used for production of a zinc-rich ingredient for functional food products.

Keywords: accumulation, growth rate, organically bound Zn, *Saccharomyces cerevisiae*, yeast, zinc.

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INTRODUCTION

As structural and functional constituents of numerous metalloproteins, enzymes and hormones, microelements are involved in several biological processes of living organisms (Berg et al. 2002). Also certain ions neutralize electrostatic forces present in various cellular anionic units, especially in the DNA double helix and cell membranes (Jones, Greenfield 1984). On the other hand, it has been demonstrated that better bioavailability and consequently more efficient absorption of organically bounded microelements are achieved when administered in the form of protein complexes and obtained from the digestive tract of humans and animals (Dobrzanski, Jamroz 2003).

Yeasts are known for their ability to bioaccumulate minerals from aqueous solutions, from which they permanently incorporate these metal ions into own cellular structures owing to their high concentration of protein (Blackwell et al. 1995, Templeton et al. 2000, Cornelis et al. 2001). Yeast cells incorporate double-valence metals through mechanisms including production of metalloproteins, mineralization and capture of metals into vacuoles (Ecker et al. 1986, Welch 1993, Eide 1997). Numerous *studies* about the processes involved in the uptake of trace metals by the yeast *Saccharomyces cerevisiae* as a model organism for investigating metal transporters and their accumulation in the cells have been conducted in recent years (Nelson 1999, Cohen 2000).

Zinc is an essential element required for *Saccharomyces cerevisiae* growth and metabolism. Also, zinc metalloenzymes are recognized in all 6 enzyme classes as identified by the IUBMB* (Valee, Falchuk 1993). Moreover, zinc is involved in the structure and function of proteins and nucleic acids, gene expression and immune system development (Castro, Sevall 1993, Dardenne, Bach 1993, Rebar, Miller 2004).

The bioaccumulation of zinc ions by yeast proceeds in two stages (Mowll, Gadd 1983). The first, known as biosorption or "passive capture", is independent of microbial metabolism and is related to the cation accumulation on the outer surface of a cell wall, so that the metals retained there are subsequently adsorbed on anionic binding sites present in the cell intramicrofibrillar structures (Volesky, May-Phillips 1995, Gaudreau 2001). The second stage, called bioaccumulation or "active capture", is metabolism-dependent intracellular uptake and involves the penetration of metal ions to the cell interior using specific membrane transporters and the cell's metabolic cycles (Brady, Duncan 1994). Metal ions are then primarily accumulated in the yeast vacuole (Macdiarmid 2002). The optimal concentration of zinc is yeast strain dependent. Generally, for *S. cerevisiae*, 0.25-0.50 mg l^{-1} appears to be required for the cell growth, and 1-2 mg l^{-1} is optimal for glycolysis (Jones, Greenfield 1984).

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Owing to its ability to incorporate metals, yeast biomass has been used frequently as a delivery vehicle for many minerals. Zinc enriched yeast is produced by growing yeast cells in Zn high medium. The uptake of zinc by yeast cells may be affected by numerous parameters including the chemistry of the metal ions, cell physiology, specific surface properties of the organisms and physicochemical influence from the environment (Godlewska-Zlkiewicz 2006). However, high concentration of zinc ions in a nutrient substrate may be toxic, since zinc affects the permeability of membranes to potassium, causing a decrease of the yeast growth (Liu 1997). Thus, it seems that an optimal concentration of Zn is critical. Few studies have been completed on the optimal concentration of Zn in yeast and its effects on yeast characteristics (De Nicola et al. 2009 *a*,*b*).

Therefore, the aim of the present study was to produce high quantities of Zn-enriched yeast biomass in the presence of this metal using an industrially important strain of *S. cerevisiae*. Other objectives were to determine the zinc *concentration* in Zn-enriched biomass by rapid colorimetric assay and atomic absorption spectrometry, and to assess the effect of different Zn concentrations in culture medium on the yeast growth rate and Zn content in yeast biomass.

MATERIAL AND METHODS

Organisms, media and culture conditions

The *Saccharomyces cerevisiae* (strain PTCC 5209) used in this study was obtained from the collection of the Industrial Microorganisms Laboratory of the Iranian Research Organization for Science and Technology (IROST). Agar plates for storing and maintaining the viability of yeast culture were prepared by addition of 15 g l^{-1} agar to the Sabouraud dextrose broth (SB).

The composition of the basal medium* (BM) for yeast cultivation was (g l^{-1}): Dextrose – 20.0; Peptic digest of animal tissue – 5; Pancreatic digest of casein – 5. The batch processes were performed in 500 ml Erlenmeyer flasks with 200 ml of BM. Cultivation was performed in a shaker agitated at 200 strokes min^{-1} at 28°C for 72 h. The pH of the medium was initially adjusted to 4.5 using HCl at 0.1M. Zinc concentrations of the SB medium were adjusted to 0, 5, 10, 15, 30 and 60 mg l^{-1}, using a 1,000 mg l^{-1} sterile stock solution of zinc sulfate heptahydrate (Merck, Germany). The time of the addition of the $ZnSO_4$ solution was 0 h of inoculation. After cultivation, samples were centrifuged at 4000 rpm for 10 min at 20°C. The supernatant fluid was discarded and the solid phase was washed with deionized water thrice in order to remove residues of the medium and surface-bound zinc.

* Sabouraud Dextrose Broth; Liofilchem, Italy

Growth rate evaluation

In the course of cultivation, the yeast growth was determined at 24-hour intervals (0, 24, 48, and 72 hours) turbidimetrically, by measuring the suspension optical density at 600 nm wavelength (OD_{600}). Also, at the end of cultivation, the dry biomass yield was determined by centrifuging 25 ml of samples at 4000 rpm for 10 min in a portable centrifuge. Immediately after centrifugation, the supernatant was removed and the content of dry matter was determined using two-stage drying, first at the temperature of 60°C for 2 h, and then finish drying at 105°C until the constant weight was reached. The yield of yeast cell biomass was expressed in g dry weight l^{-1} medium (NOWAK et al. 2005).

Measurement of metal content in yeast cells

After the fermentation was performed, two methods were applied for the assessment of the total zinc accumulation: colorimetric assay and ICP-MASS assay.

Colorimetric assay for zinc biosorption evalution

The concentrations of zinc in supernatant were determined spectrophotometrically (UV-visible S2100, Scinco, Korea) by using commercial kits (Giesse diagnostics, Italy).

The amount of Zn^{2+} adsorbed by cells was calculated as follows:

$$q\ (\mu g\ g^{-1}\ \text{of yeast}) = 1000\ (c_0 - c_t) \cdot V\ W^{-1}$$

where: t – the incubation time (h),

c_0 – the initial concentration of $ZnSO_4$ at t = 0 (mg l^{-1}) before inoculation of yeast,

c_t – the concentration of residual Zn^{2+} in supernatant at the time of t (mg l^{-1}) after removal of biomass at the end of cultivation,

V – the volume of medium (ml),

W – the dry weight of yeast (g).

In addition, yeast cells were washed with ddH_2O to eliminate residues of Zn^{2+} adsorbed to the surface of the biomass, and amount of surface-bound Zn was determined by the above method.

Zn Incorporation Efficiency: The final Zn content (mg) in the produced yeast was divided by the initial amount of $ZnSO_4$ (mg) added to the medium and multiplied by 100.

Determination of total accumulated zinc in yeast cells

The content of zinc in whole yeast cells was assessed according to the modified AOAC method based on YANG et al. (2005). Briefly, dry yeast biomass was digested by the addition of 5 ml of 65% (v v^{-1}) nitric acid* to 1 mg dry biomass in 25 ml digestion tubes. The pre-digested samples were heated for 1 h at 90°C, then the temperature was raised to 140°C and hydrogen peroxide** (30%) was added to the tubes. After cooling down, the samples were diluted with bidistilled water up to 10 ml and the zinc content was analyzed by ICP-MASS (Yokogawa Analytical Systems HP 4500, Japan).

Zinc standard solutions were prepared by using appropriate dilutions of a stock zinc standard solution for ICP-MASS analysis (1000 ± 2 mg $ZnSO_4$ l^{-1} in 5% HNO_3, Merck). The amount of total zinc was calculated per gram of dry weight (μg Zn g^{-1} d.w. of yeast).

Moreover, different forms of Zn (organic and inorganic forms) present in the biomass were quantified as described by ROEPCKE et al. (2011).

All glassware was washed in 10% HCl*** and rinsed 3 times with distilled water to avoid any mineral contamination.

Statistical analysis

The experiment was carried out in a completely randomized design with 4 replications. Each variable was compared using the Anova procedure of SAS (1996) to determine statistical significance. Differences among treatment means were separated using the Duncan's multiple range test. Verification of significance was based on $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth rate assay

The effects of supplementing the culture medium with zinc sulfate on the yeast growth rate are *illustrated* in Figure 1. As expected, the growth rate was higher up to 24 hours after inoculation but lower in comparison to 0-24 hour inoculums (P<0.05). Also, the OD of the cell culture increased correspondingly with the incubation time and leveled off at around 60 h after inoculation, indicating the end of the yeast cell growth as the number of yeast cells present in the broth remained constant (Figure 1).

The above trend was in agreement with the growth curve for yeast cells reported by other investigators (DALGAARD, KOUTSOUMANIS 2001). According to VASANTHY (2004), the growth rate of yeast biomass rose with the incubation

* HNO_3 65%, Merck, Germany
** H_2O_2 30% Merck, Germany
*** HCl 37% Merck, Germany

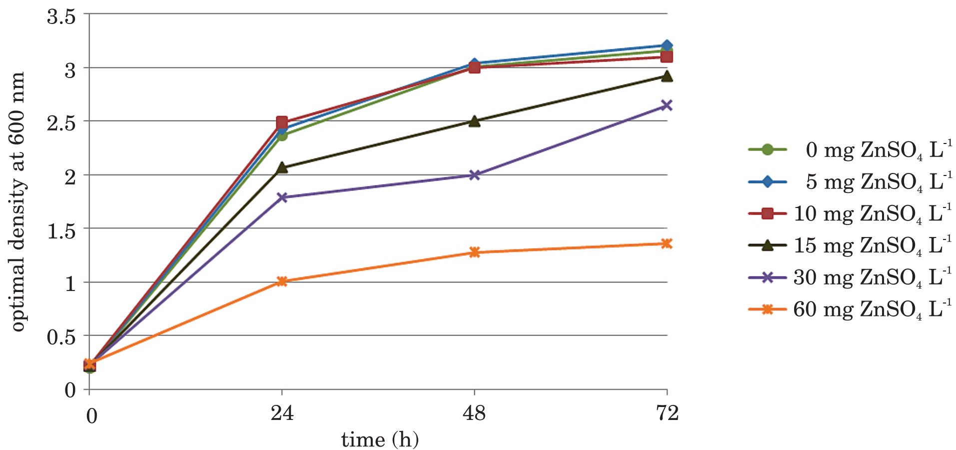

Fig. 1. The effect of zinc sulfate on the growth of *S. cerevisiae* yeast

time, reached the maximum at an equilibrium time and afterwards remained constant.

A similar upward trend in the yeast growth rate was found at 24, 48, and 72 hours after inoculation across treatments. The continuation of the growth rate was consistent and dose related. However, in the presence of 5 and 10 mg l^{-1} of $ZnSO_4$, the growth curves and the duration of the lag-period were almost the same as in the control culture (Figure 1). Furthermore, the concentration of $ZnSO_4$ 5 mg l^{-1} resulted in a slight OD_{600} increase in the growth phase. As the $ZnSO_4$ concentration rose up to 15 mg l^{-1}, a decrease in the growth rate during the cultivation was observed. Higher $ZnSO_4$ concentrations, i.e. 30 and 60 mg l^{-1}, more drastically inhibited the yeast growth rate during the linear phase (P<0.05). The maximum OD for these treatments did not exceed 43% of that in the control culture.

Zinc added to culture medium Zn may enhance the cell growth yeast (Jones, Gadd 1990) because this element is involved in the regulation of structure and metabolic activity of yeast and other processes such as flocculation and cell division (Bromberg 1997).

Our results show that the addition of $ZnSO_4$ up to 5 mg l^{-1} of nutrient media slightly increased the growth of *S. cerevisiae*. This growth stimulating effect of Zn in low concentrations can be explained by the increased synthesis of riboflavin and stimulation of cell proliferation, which lead to a more intensive cell growth (Greco et al. 1990). However, the growth rate of yeast cells and the final biomass yield were reduced drastically by increasing the $ZnSO_4$ concentration above 10 mg l^{-1} of medium. The inhibitory effect of high concentrations of Zn salts in nutrient media on the growth of yeast cells and/or on the final biomass yield is due to the harmful effects caused by the toxic nature of zinc. Zinc toxicity inevitably results in the biological inactivation of cells and loss of viability.

Since toxicity depends on the Zn concentration, its manifestation in metabolism is proportional to the amount of zinc capable of permeating into the cell and interfering with its biological functions. On the other hand, an increase in the $ZnSO_4$ concentration lengthened the lag-phase and shortened the exponential phase duration, thereby decreasing the yield of yeast biomass. These results are consistent with the observation of KONOPKA et al. (1999), who confirmed that the microbial biomass generation diminished as the concentration of the heavy metal increased.

It can be assumed that the presence of large amounts of Zn ions taken up by *S. cerevisiae* causes extensive K^+ release, as well as Mg^{2+}, Na^+ and H^+ efflux from the biomass, which may be an essential component of the physiological mechanism responsible for maintaining the ionic balance, or may be a symptom of membrane disruption and irreversible viability loss (PASSOW, ROTHSTEINA 1960, NORRIS, KELLY 1977, GADD, MOWLL 1983).

Table 1

Yields of yeast biomass and accumulation of zinc in biomass of *S. cerevisiae* yeast with the addition of different concentrations of $ZnSO_4$ into medium

Treatment*	Yeast biomass yield (g l^{-1})	Zinc accumulation (μg g^{-1} of yeast)		
		total Zn accumulation	organically bound Zn	inorganically bound Zn
1	4.342^{b}	171.8^{e}	163.2^{e}	8.590^{f}
2	4.995^{a}	1888.5^{d}	1756.4^{d}	132.2^{e}
3	4.260^{bc}	3265.9^{c}	3004.7^{b}	261.2^{d}
4	3.863^{c}	3795.7^{b}	3302.3^{a}	493.4^{c}
5	3.020^{d}	4132.3^{a}	3140.5^{ab}	991.8^{b}
6	1.741^{e}	3895.9^{ab}	2415.5^{c}	1480.5^{a}
P value	0.001	0.001	0.001	0.001
SEM	0.260	33.32	27.54	7.390

Means with same superscripts in the same column are not significantly different ($P<0.05$).
SEM: Standard error of the means.
* Treatment: 1 – without the addition of zinc (control); 2–5; 3–10; 4–15; 5–30; 6–60 mg zinc l^{-1} of medium.

Moreover, one of the major Zn detoxification mechanisms in *S. cerevisiae* is the induced synthesis of metallothioneins (MT), which restrains the binding of free Zn ions in the cytosol (WELCH et al. 1983). Metallothioneins can be induced by heavy metal ions, such as Zn, Cu, Cd and Co. However, the MT in *S. cerevisiae* can only be induced by Cu (VIJVER et al. 2004), hence it iis called Cu-MT. Therefore, the absence of MT synthesis and consequently loss of Zn homeostasis under high Zn concentrations result in toxicity and retarded cell growth.

In terms of biomass production, the results shown in Table 1 demonstrate that as the $ZnSO_4$ concentration increased above 15 mg l^{-1} in the medium, the biomass yield significantly diminished ($P<0.05$), whereas an

increase in the concentrations of $ZnSO_4$ in a medium from 0 to 10 mg l^{-1} had no influence on the yeast biomass production (P<0.05). The minimum and maximum biomass production was 4.99 and 1.74 mg l^{-1} biomass in media containing $ZnSO_4$ at concentrations of 5 and 60 mg l^{-1}, respectively (Table 1).

Excessive $ZnSO_4$ (above 15 mg l^{-1}) appeared to have a detrimental effect on the yeast growth. HAMMOND (2004) reported that excessive Zn could depress the growth of brewing yeast unless the concentrations of manganese were correspondingly high. Therefore, the results indicate that although Zn is an essential element for *S. cerevisiae*, Zn^{2+} added in high concentrations had inhibitory effects on the yeast cell growth.

The results of the current study suggest that zinc ion concentrations in medium are not a sufficient condition for achieving an ideal yeast biomass yield because adding low concentrations of $ZnSO_4$ (5 mg l^{-1}) promoted the yeast cell growth.

Colorimetric assay for zinc biosorption evaluation

The magnitude of biosorption was significantly different (P<0.05) in the presence of different concentrations of the initial $ZnSO_4$ in media (Figure 2). Except for $ZnSO_4$ concentrations exceeding 60 mg l^{-1}, higher $ZnSo_4$ supplementation resulted in enhanced zinc biosorption, so that the maximum Zn^{2+} biosorption was recorded at the initial zinc concentration of 30 mg l^{-1}

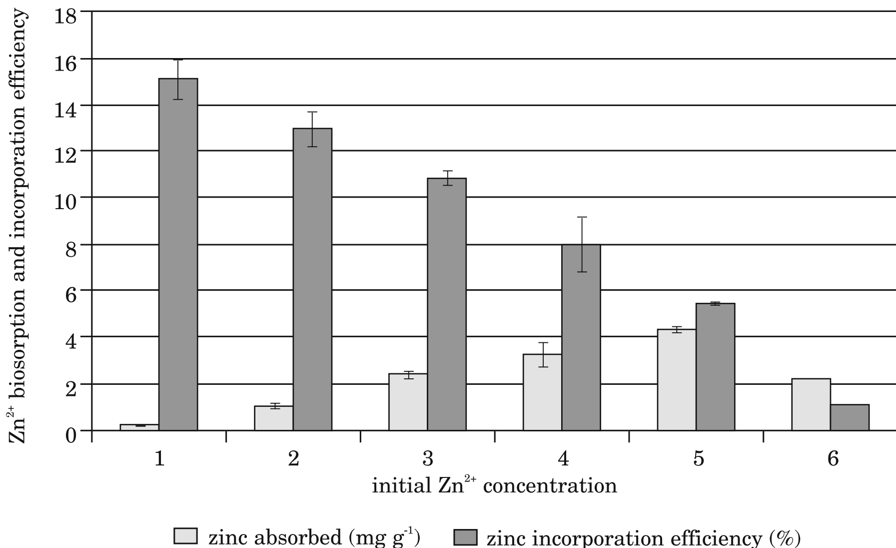

Fig. 2. Effect of initial Zn^{2+} concentration on zinc biosorption (mg g^{-1} of yeast) and Zn^{2+} incorporation efficiency (%)

Treatment: 1 – without the addition of zinc (control); 2–5; 3–10; 4–15; 5–30; 6–60 mg zinc l^{-1} of medium.

Note: Error bars denote standard deviation. Statistical significance by *t* test: p<0.05.

(4.33 mg g^{-1} of yeast) and the control culture contained the lowest amount of Zn (0.23 mg g^{-1} of yeast).

The introduction of Zn salts into culture medium results in the uptake of this mineral by yeast cells. The uptake of Zn^{2+} by *S. cerevisiae* requires the presence of functional groups such as the phosphomannan in their cell walls, the availability of free carboxyl, amine, phosphate, hydroxyl and hydrosulphide groups in the surface proteins and an intact membrane (BLACKWELL et al. 1995). As the amount of $ZnSO_4$ in the growth medium increased, the ability of yeast cells to adsorb it decreased due to the *saturation* of functional groups on the surface of the cellular walls and the *binding sites and* chelating agent within cells.

Moreover, the reduced Zn^{2+} adsorption due to the addition of 60 mg l^{-1} Zn sulfate to a medium may be explained by the fact that the Zn^{2+} uptake into cells is mediated by the plasma membrane ATPase activity via the transmembrane proton gradient (PONTA, BRODA 1970). Thus, the uptake of the metal usually takes place during the early stages of fermentation, when the availability of energy sources is the highest. Nevertheless, the cells of *S. cerevisiae* released some of the previously bound Zn^{2+} into the medium at the stationary growth stage to prevent the cell from Zn supersaturation and toxicity. It was reported that the mass of incorporated Zn ions was reduced towards the end of fermentation as cells released the metal ions due to the ageing process and reduction in the cell's charge (WALKER 2004).

The results indicated that the Zn^{2+} uptake capacity (q, μg g^{-1}) increased and the Zn^{2+} incorporation efficiency decreased when the initial external $ZnSO_4$ concentration in the nutrient medium was raised (Figure 2), although a 12-fold higher initial $ZnSO_4$ concentration in the yeast growth medium did not result in 12-fold higher total biosorption of Zn yeast cells after 72-h cultivation. This result might be explained by a smaller cell size due to a shorter exponential phase duration and hindered transport across the yeast cell membranes (ARSLAN 1987, HUGHES, POOLE 1991), since metal ions in the medium would not be adsorbed to the surface of the biomass, could not enter into intracellular organelles and be stored in vacuoles. The Zn^{2+} incorporation efficiency ranged from 1.19% to 15.01% when the initial supplemental $ZnSO_4$ concentration is 0-60 mg l^{-1}. The above percentages occurred in response to the highest initial $ZnSO_4$ concentration (60 mg Zn l^{-1}) and control culture, respectively.

ICP-MASS assay for Zn accumulation evaluation

Table 1 illustrates the quantity of total Zn accumulated by yeast cells. Except for the concentration of 60 mg l^{-1} $ZnSO_4$ added to the medium, the content of Zn accumulated by the yeast biomass was proportional to the $ZnSO_4$ concentration of the medium ($P<0.05$), namely after 72 h of incubation, the highest Zn bioaccumulation by yeast biomass was obtained in a medium supplemented with 30 mg l^{-1} $ZnSO_4$ ($P<0.05$), and the Zn content in

that biomass reached 4132 μg g^{-1} of d.w. Also, the control culture had the lowest concentration of Zn in its biomass at 171 μg g^{-1}.

The direct relationship between the Zn content in cells and Zn supplemented in the culture medium indicates the cell's inability to curb zinc accumulation even at lethal doses. Nevertheless, this dependence was not linear (Table 1). Thus, under the increase in the exogenous $ZnSO_4$ concentration up to 30 mg l^{-1}, the Zn bioaccumulation increased. In response to higher $ZnSO_4$ concentrations supplemented, the concentration of accumulated Zn slightly decreased. Production of zinc-enriched yeast biomass has been reported by some other authors. STEHLIK-THOMAS et al. (2004) obtained the total concentration of Zn of 700 μg g^{-1} of dry biomass by the addition of 100 mg l^{-1} $ZnSO_4$ to the medium, while ŠILLEROVÁ et al. (2012) produced yeast biomass enriched with 3820 μg Zn g^{-1} of *S. cerevisiae* biomass through yeast cultivation in medium supplemented with 250 mg l^{-1} $ZnSO_4$.

Based on some previous study in hypertonic solutions, it can be concluded that microbial cells tend to shrink and reduce their volume. Depending on the osmotic stress, they may lose as much as 60% of their initial volume (GNIEWOSZ et al. 2006), and the maximum Zn bioaccumulation capacity by cells appeared to depend on cellular volumes (DE NICOLA et al. 2009*b*). On the other hand, the vacuole is the major site of metal storage in the *S. cerevisiae* cell, and Zn is translocated predominantly into the yeast vacuole (DE NICOLA et al. 2009 *a*).It is therefore conceivable that yeast with smaller cellular volumes and consequently smaller vacuoles are able to accumulate less Zn than larger cells. Also, the ability of cells to accumulate Zn at higher concentrations could depend on other factors, such as the presence of Zn-binding intra-vacuolar components, e.g. polyphosphate bodies (JONES, GADD 1990). Moreover, the size of the intracellular pool determines the activity of permease, a membrane transport protein that inhibits the uptake of a substance by trans-inhibition, so that at higher pool levels, more permease molecules are inactivated (FAILLA, WEINBERG 1977).

In addition, the production of Zn-enriched yeast depends not only on the Zn content in cells, but also on its amount in biomass. A high Zn content in cells was accompanied by a low biomass and vice versa. This result is in conformity with the report by DUSZKIEWICZ-REINHARD et al. (2005) on the binding capacity of magnesium in yeast.

Organically and inorganically bound zinc

Fractions of Zn accumulated in the biomass were also determined (Table 1). The amount of accumulated organically bound Zn was higher than inorganically bound Zn fractions in all thee treatments. Based on these results, the intracellular Zn content in yeast was in the range of 62% to 95% of the total Zn in cells. The control culture containing 171 μg Zn g^{-1} of biomass had 95% of organic bound zinc, which means that the yeast product was relatively free of inorganic Zn. On the other hand, T6 (60 mg l^{-1} $ZnSO_4$ - supplemen-

ted) contained 3895 μg Zn g^{-1} of yeast biomass, and 38% of the total Zn was present in the inorganic form. Moreover, the yeast cells accumulated up to a 20-fold higher amount of organically bound Zn with the 15 mg l^{-1} of $ZnSO_4$ in the medium compared to the control culture.

Organically bound Zn is the zinc fraction bound to organic molecules present in the cell. The higher $ZnSO_4$ concentrations resulted in enhanced intracellular Zn accumulation, evidently due to the Zn sorption by cell wall polysaccharides and intracellular organelles. Accumulation of a significant amount of Zn in extracellular polysaccharides has been observed by other researchers (ROEPCKE et al. 2011).

After absorbtion of Zn by yeast cells, metal ions are compartmentalized into different subcellular organelles (e.g. mitochondria and vacuoles), and this mineral combines with many intracellular organelles including cell wall, cell membrane, vacuoles and mitochondria. At excess Zn concentrations, vacuoles uptake and store high amounts of Zn to maintain the normal metabolism of cells (DANIEL et al. 19990), bind this mineral with a chelating agent and accumulate them in the organic form.

As mentioned in the previous section, restriction of Zn-binding ligands within intracellular organelles including polyphosphate and organic anions, such as glutamate and citrate, may contribute to the reduction of Zn bioaccumulation capacity under high initial concentration of this salt (KITAMOTO et al. 1988).

The results obtained from the ICP-MASS assay are partly consistent with measurements of the total Zn biosorption in yeast cell determined by colorimetry. However, the lack of strong similarity between these analytical methods is probably explained by the characteristics and limitations of colorimetric assays and weak surface binding of Zn in yeast cells.

CONCLUSION

The experimental results justify the conclusion that the concentration of Zn sulfate in the culture medium significantly affected the Zn content in yeast cell as well as the cell growth. Thus, the addition of Zn salts into a yeast (*S. cerevisiae*) medium enhances organic Zn production. The enhancement was higher under 30 mg l^{-1} $ZnSO_4$ levels and the highest level of zinc sulfate (60 mg l^{-1}) limited the yeast growth and Zn uptake. Therefore, addition of $ZnSO_4$ to a medium for yeast cultivation in a concentration higher than 30 mg l^{-1} is unreasonable because it inhibits the growth of yeast and causes accumulation of Zn in inorganic forms, which is unacceptable for production of a zinc enriched ingredient for food products. It seems that the former concentration of $ZnSO_4$, above which growth inhibitions occurs, is optimal for the production of Zn enriched yeast biomass. Moreover, the

colorimetric assay of Zn was shown to be a fairly reliable and efficient tool for assessment of the Zn content in future studies.

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