Journal of Elementology ISSN 1644-2296

Szulc-Musioł B., Dolińska B., Ryszka F. 2019.
Effect of culture parameters on selenium accumulation in Saccharomyces cerevisiae cells.
J. Elem., 24(1): 155-164. DOI: 10.5601/jelem.2018.23.2.1574

RECEIVED: 12 December 2017
ACCEPTED: 8 August 2018

ORIGINAL PAPER

EFFECT OF CULTURE PARAMETERS ON SELENIUM ACCUMULATION IN *SACCHAROMYCES CEREVISIAE* CELLS

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Abstract

Selenium may play a beneficial role in multi-factorial diseases with genetic and environmental linkages via regulation selenoproteins activity. The supply of food supplemented with Se-enriched yeast is one of the ways to overcome deficiency of this microelement. The effect of incubation conditions on selenium binding by *Saccharomyces cerevisiae* was studied. The study process factors were as follows: kind of sugar (glucose, sucrose, fructose, lactose, maltose) and selenium concentration (0.1, 0.3, $0.7 \cdot 10^{-3}$ mol dm^{-3}). The cultures were incubated for 60 min in a shaker incubator at a temp. 30°C. The content of selenium in the yeast was determined by the fluorometric method. It was demonstrated that the yeast intracellular selenium concentration increased and the yeast dry biomass yield decreased along with an increased selenium concentration in the culture medium. The highest biomass production was obtained in the media containing glucose, while the lowest was in the media containing lactose; depending on the conditions of incubation, the accumulation of selenium into the yeast cells was in the range of 54.29-692.88 mg kg^{-1} of dry biomass. The highest amount of selenium, that is 692.88 mg kg^{-1} of dry biomass, was bound from the medium by baker's yeast cultured in the presence of glucose and $0.7 \cdot 10^{-3}$ mol dm^{-3} concentration of selenium. Under these cultivation conditions, almost no-waste yeast production was obtained – with the lowest amount (about 11.53%) of the remaining, i.e. unincorporated, selenium in the culture medium.

Keywords: yeast; *Saccharomyces cerevisiae*; selenium; cultivation conditions; biomass yield.

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INTRODUCTION

Selenium is an essential trace element and a key component of several enzymes and proteins involved in antioxidant defense and many metabolic processes at the cellular level. Selenium supplementation may prevent various diseases and also alleviate other pathological conditions, including oxidative stress and inflammation (Papp et al. 2007, Weekley, Harris 2013, Selenius et al. 2010). In addition, selenium and selenoproteins have been reported to participate in the immunological function and sperm maturation (Mehdi et al. 2013).

Since the publication in 1996 of the *Saccharomyces cerevisiae* genome sequence, the yeasts have become a model organism in the molecular biology of eukaryotes. They have many advantages: they are non-toxic, non-pathogenic and do not require high cost of culture maintenance

Moreover, they are characterized by a high content of proteins with a favourable amino acid profile, and the ability to grow on various substrates. Owing to these features, *Saccharomyces cerevisiae* are microorganisms readily used on an industrial scale in the production of foodstuffs and feeds, in biotechnology and pharmacy. They are also applied to manufacture protein preparations and dietary supplements (Podpora et al. 2015).

Yeasts can bind ions of elements from the environment, incorporating them into their protein structure, where they serve as essential enzyme cofactors. Depending on the incubation conditions, baker's yeast can accumulate copper, iron, manganese, selenium, chromium, zinc and iodine compounds (Dolińska et al. 2006, 2009, Ryszka et al. 2013). Unfortunately, these elements may also become toxic in higher doses. The bioavailability and toxic effect of selenium depend not only on the administered dose, but also on the chemical form of the element (Thiry et al. 2012). An efficient way to overcome selenium deficiency is to provide selenium-enriched food, especially selenium-enriched biomass with organic forms of the microelement. Organic selenium complexes and seleno-amino acids are considered to be much more bioavailable and effective forms of selenium than inorganic salts (Fairweather-Tait et al. 2010). Pharmaceutical preparations and food including in their composition selenium bound to yeast are stable for 2 to 3 years and selenium does not interact with other ingredients (Szulc-Musioł et al. 2003, Rayman 2004). It has been proven that baking does not have any significant impact on selenium stability in bread. Thus, selenium yeast would be suitable for other food products, particularly those in a powder or liquid form (Esmaeili, Khosravi-Darani 2014).

Based on the *Saccharomyces cerevisiae* cultures and molasses, a waste-free technology for the production of Biocer® food yeasts, enriched in selenium, zinc and chromium has been developed. In the bioavailability studies of laying hens receiving Biocer® feed yeast, higher assimilability of selenium and zinc was demonstrated, in comparison with the control

group whose diet included inorganic forms of these elements (Na_2SeO_3, ZnO) – Dobrzański et al. (2003). The supplementation of laying hens with feed containing iodine-enriched yeast at a dose of 2 mg kg^{-1} of feed for 12 weeks (Opalinski et al. 2012) caused a significant increase in egg and albumen weight and about 90% increase in iodine content in egg yolk, in comparison with the group of birds fed with the inorganic form of that element commonly used in animal feeds. In digestibility-balance studies, it was found that yeasts enriched with copper and manganese may be used in practice for the feeding of pigs (Korniewicz et al. 2007).

Production of yeast biomass containing organic selenium stems from the research on finding a method of dietary supplementation with selenium (Dobrzański et al. 2002, 2004, Szulc-Musioł et al. 2016).

The aim of the study was to establish the efficiency of selenium accumulation by using yeast cells from a culture medium containing different concentrations of sodium selenite and various carbon sources (glucose, sucrose, fructose, lactose, maltose).

MATERIALS AND METHODS

Chemicals

Fresh *Saccharomyces cerevisiae* baking yeast, light beige incolour, having solid consistency and the dry mass content of 33% (Lesaffre Bio-Corporation, Wołczyn, Poland) was tested. Anhydrous glucose, sucrose, fructose, lactose, maltose were purchased from Avantor Performance Materials Poland S.A. All the reagents were of analytical grade or higher purity. A Milli-Q (Millipore) system was used to obtain water applied in the experiment.

Experimental procedure

Crumbled yeast (15 g of fresh, 5 g of DM) was added to 0.2 dm^{-3} of aqueous sugar solutions at the concentration of 2% and stirred up for 10 min with a magnetic stirrer. Fermentation was carried out with different carbon sources, including glucose, sucrose, fructose, lactose, maltose. Then, working solution of selenite was added in such volumes that the final selenium concentrations in the experimental media were 0.1, 0.3 and 0.7 $\cdot 10^{-3}$ mol dm^{-3}. The mixture was again stirred up carefully until a homogenous suspension was obtained. Cultures were grown in 1000 ml flasks on a reciprocal shaker (KS 15 Controler Edmund Buhler, GmbH), at an amplitude of vibration equal to 180 cycles min^{-1} at 30°C for 60 minutes. The pH of the media was maintained at 5.0. Following the incubation, the samples were harvested by centrifugation at 10 000 x g for 10 min. The biomass was suspended twice in 200 ml deionized water, stirred up for about 5 min, and then centrifuged again. The wet sediment was initially dried at a temp. of 60°C for 2 hours. Next,

the process of drying up was continued at 105°C, until the constant weight was obtained.

Selenium assay procedure

The method for selenium determination has been described previously by Danch and Dróżdż (1996). Briefly, 10 mg samples were mineralized in perchloric acid solution in a combustion apparatus (Buchi DigestionUnit K-435 Germany). Selenates (SeVI), obtained as a result of mineralization, were reduced to selenites (SE IV) using hydrochloric acid. Then, the selenites were complexed with 2,3-diamino-naphthalene and the resulting product (4,5-benzopiazoselenol) was extracted with cyclohexane. Selenium in the organic phase was determined spectrofluorimetrically with the use of a Perkin-Elmer apparatus, setting excitation and emission wavelengths 356 and 520 nm, respectively.

Statistical analysis

Statistical analyses were performed using Statistica software 9PL. The results were represented as the mean of five experiments (x). Standard deviation (SD) was calculated. Total process efficiency of the yeast biomass obtained was calculated according to the following formula:

$$\text{total process efficiency (\%)} = \frac{\text{content selenium in culture medium (mg)}}{\text{the amount of built} - \text{in selenium in (mg kg}^{-1}\text{ DM)}} \cdot 100\%$$

The statistical significance of the differences between the means was analyzed using the Kruskal-Wallis test. A level of $p \leq 0.05$ was adopted to indicate statistical significance.

RESULTS AND DISCUSSION

Baker's yeast is a source of protein containing endogenous amino acids and enzymes, B vitamins and many other valuable biologically active compounds. It can also be a good source of β-glucans or mono- and oligosaccharides (Jarmołowicz et al. 2013) although it is not a good source of minerals. *Saccharomyces cerevisiae* show the capability of binding elements present in the environment often in amounts considerably exceeding their physiological demand. Metal ions adsorbed on the cell's surface may next be a subject of intracellular bioaccummulation. This way yeasts produce metal-protein complexes called bioplexes.

Optimization of the conditions which affect selenium yeast production has been the aim of many studies (Ponce de León et al. 2002, Kieliszek et al. 2016, Kaur, Bansal 2006, Suhajda et al. 2000).

Saccharomyces cerevisiae do not contain selenoproteins and therefore selenium is not essential for these organisms (Lu, Holmgren 2009). When selenium feeding is not carefully controlled, selenium can be toxic to yeast cells since it generates oxidative stress and even provokes DNA damage (Lewinska, Bartosz 2008, Letavayová et al. 2006). In turn, oxidative stress occurrence in yeast cells induces the expression of genes responsible for the biosynthesis of selenoproteins. Kieliszek et al. (2016) observed fractions of protein of yeast obtained from media supplemented with selenium (20-40 mg L^{-1}), and detected the presence of four new protein fractions compared to electropherogram proteins from control cultures.

It was demonstrated in previous studies on *Saccharomyces cerevisiae* yeast that the sugar concentration in a growth medium significantly influenced the biomass yield (Dobrzański et al. 2004). At a lower sugar content (2%), the biomass was obtained with the efficiency of 93.5%. When the culture medium contained 4.7% of sugar, the biomass yield was 25% lower than in the case of culture containing its smaller amount. The yeast content in the culture medium also significantly influences the biomass yield (Dobrzański et al. 2004). When the yeast content amounted to 6%, the yeast biomass efficiency was 90.73%. When the yeast content was higher, i.e., 12%, the biomass yield was 22% lower. Lower yeast and sugar content in the culture medium is also advantageous because it eliminates the problem of residue neutralization and facilitates the drying process of the final product.

In the present work, *Saccharomyces cerevisiae* yeast was incubated in a medium culture with different types of sugar (glucose, sucrose, fructose, lactose, maltose) and with an inorganic form of selenium, i.e., as sodium selenite, at various selenium concentrations (0.1, 0.3, $0.7 \cdot 10^{-3}$ mol dm^{-3} selenium), at 30°C for 60 min.

Figure 1 presents the effect of various types of sugar and selenium concentrations on yeast biomass production. It could be concluded that the selenium concentration in the yeast growth medium affects the yeast dry biomass yield. *Saccharomyces cerevisiae* yeast biomass after fermentation constituted between 7.02 and 9.82 g. The highest biomass production was obtained in the media containing glucose. The maximum yeast biomass (9.82 g) was obtained in variant 1, with the glucose concentration of 2% and the selenium concentration in the medium of $0.1 \cdot 10^{-3}$ mol dm^{-3}. Under these conditions, the highest total process efficiency was observed, reaching 88.47%. Numerous studies have confirmed that glucose is a preferred sugar in fermentation metabolism (Ponce de León et al., Suhajda et al. 2000). Yields of biomass obtained from the culture media containing lactose were lower than amounts produced from the media containing other sugars. The carbon sources that supported yeast biomass production can be arranged in this order: glucose< sucrose< maltose< fructose <lactose. A decrease in yeast biomass was dose-dependent relative to the an increasing selenium concentrations in the culture medium.

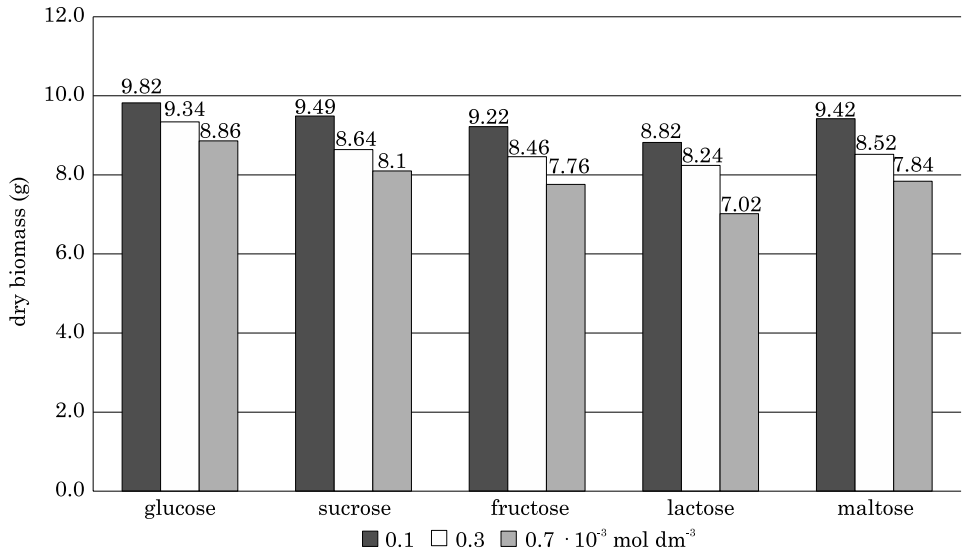

Fig. 1. Effect of the kind of sugar and selenium concentration on yeast biomass production

The biomass yield of selenium yeast cultivated in the medium containing $0.7 \cdot 10^{-3}$ mol dm^{-3} of selenium was significantly lower ($p < 0.05$), compared to the biomass yield when the selenium was added to the medium at the dose of $0.1 \cdot 10^{-3}$ mol $dm^{-3.}$

The effect of incubation conditions on the process of selenium incorporation into *Saccharomyces Cerevisiae* yeast is shown in Table 1. In the process of intracellular accumulation depending on the culture conditions, selenium may be organically bound and/or reduced to elementary selenium, which gives a reddish colour to the yeast cells. The colour of the yeast biomass grown in the media in all the samples was drab, which indicated that little elemental selenium accumulated in the biomass.

Both the type of sugar and the concentration of selenium in the growth medium significantly influence ($p < 0.001$) selenium content in baker's yeast cells. The effect of increasing selenium concentration in experimental medium on its accumulation in the biomass was relatively proportional and differences between the individual doses were statistically significant. The highest selenium content in the dried bioplex (692.88 mg kg^{-1} dry weight) was obtained in variant 3 and it was approx. 12.76 times higher than the content of selenium in variant 7. In the same conditions, the highest total efficiency of the ready-made product was obtained, i.e. about 88.47%, at an almost no-waste yeast production, with the lowest amount (about 11.53%) of the remaining, i.e., unincorporated, selenium in the culture medium.

Cells did not absorb all ions present in the culture medium, even in the case of their low concentration. Accumulation of metal ions depends

Table 1

Effect of incubation conditions on the process of selenium-enriched yeast production

Variant	Kind of sugar	Selenium concentration ($\cdot 10^{-3}$ mol dm^{-3})	Biomass yield (%)	Selenium content (mg kg^{-1} of dry biomass) in yeast	Statistical significance
V-1	glucose	0.1	98.2	142.35	
V-2	glucose	0.3	93.4	372.22	$p \leq 0.05$ *vs.*V-1
V-3	glucose	0.7	88.6	692.88	$p \leq 0.01$ *vs.*V-1
V-4	fructose	0.1	92.2	113.17	
V-5	fructose	0.3	84.6	248.88	$p \leq 0.05$ *vs.*V-4
V-6	fructose	0.7	77.6	561.66	$p \leq 0.01$ *vs.*V-4
V-7	lactose	0.1	88.2	54.29	
V-8	lactose	0.3	82.4	102.58	$p \leq 0.05$ *vs.*V-7
V-9	lactose	0.7	70.2	307.24	$p \leq 0.01$ *vs.*V-7
V-10	maltose	0.1	94.2	130.34	
V-11	maltose	0.3	85.2	321.43	$p \leq 0.05$ *vs.*V-10
V-12	maltose	0.7	78.4	641.24	$p \leq 0.01$ *vs.*V-10
V-13	sucrose	0.1	94.9	123.26	
V-14	sucrose	0.3	86.4	263.45	$p \leq 0.05$ *vs.*V-13
V-15	sucrose	0.7	81.0	573.42	$p \leq 0.01$ *vs.*V-13

probably on the intracellular transportation systems and on their chelating strength by the medium's compounds and cellular substances (Tuszyński, Pasternakiewicz 2000).

Based on the results, it can be assumed that consumption of selenium by yeast probably occurs in the form of non-specific accumulation associated with the transport of selenium ions complexed with an assimilable substrate. This effect of a complexing agents was particularly visible in the case of glucose and lactose.

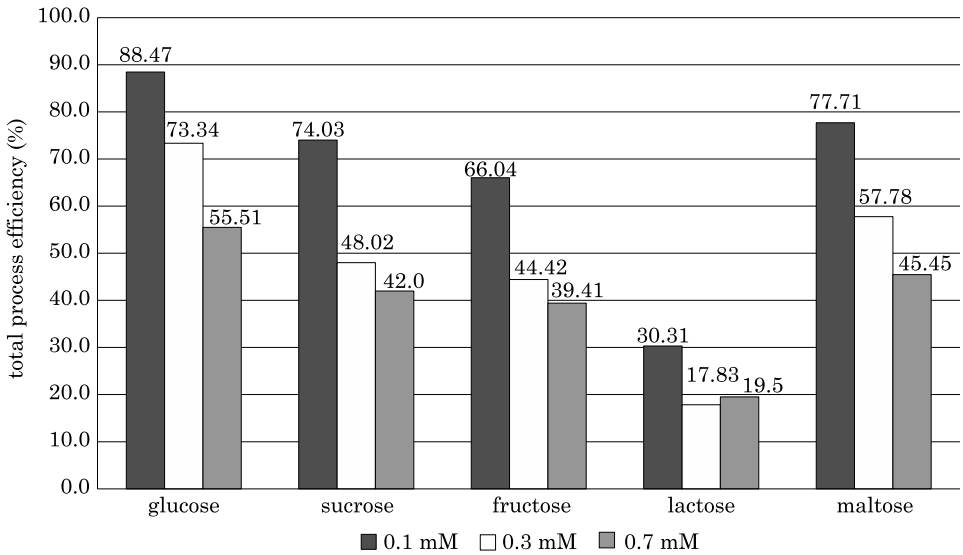

Fig. 2. Effect of the kind of sugar and selenium concentration on total process efficiency

CONCLUSIONS

The aim of the study was to elaborate simple procedures with the use of cheap materials in the process of baker's yeast enrichment with selenium.

The use of different kind of sugars and selenium concentrations in a medium culture resulted in the accumulation of selenium into the yeast cells in the range of 54.29-692.88 mg kg^{-1} of dry biomass.

In this study, increasing the amount of added selenium increases the inhibition of yeast cell growth, even though the total selenium concentration in yeast cells increases with the increased addition of selenium to the cultivation media. The increase in yeast biomass depending on the carbon source can be arranged as follows: glucose>sucrose> maltose> fructose >lactose.

Our study revealed that an addition of glucose and $0.7 \cdot 10^{-3}$ mol dm^{-3} selenium concentration in a culture medium, as well as its short-time exposition, seem to be the most effective method of obtain selenium-enriched yeast at the lowest amount (about 11.53%) of the remaining unincorporated selenium in the culture medium. The incubation conditions described above could be used in industrial production to obtain biomass of yeast containing organically bound selenium.

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