DE GRUYTER OPEN

10.1515/aucft-2017-0011

THE EFFECTS OF ZnO NANOPARTICLES IN COMBINATION WITH ALCOHOL ON BIOSYNTHETIC POTENTIAL OF *SACCHAROMYCES CEREVISIAE*

– Research paper –

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Abstract: This paper reports about experimental results concerning the influence of 30 nm ZnO nanoparticles on biomass, carbohydrates, β-glucans, proteins accumulation and catalase enzyme activity at *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain exposed to alcohol action. Alcohol in concentrations of 2%, 5% and 10% added to culture medium has been reported to stimulate β-glucans biosynthesis and to inhibit protein synthesis. Low biomass production, with 71% less that control, was detected in the experiments with 10% alcohol. ZnO nanoparticles in combination with alcohol do not offer sufficient protection for the proteins biosynthesis, but efficiently protect the carbohydrates and β-glucans biosynthetic processes, which contents in the biomass are with 16.6% and 19.9% higher than control, respectively. The maximum value of β-glucans content was established in case of cultivation of selected yeast strain on YPD medium supplemented with 5 mg/L nanoparticles ZnO and 2% alcohol. The obtained results allowed the elaboration of new procedure for directed synthesis of β -glucans that contributed to an increase of this component with 30.7%, compared to control.

Key words: *Saccharomyces cerevisiae*, nanoparticles, alcohol, biomass production, carbohydrates, β-glucans, proteins, catalase activity.

INTRODUCTION

Nowadays, the influence of nanoparticles on different biological objects, inclusive microorganisms, is of great scientific interest (Espitia et al., 2012; Egorova et al., 2016). The study of the impact of different nanoparticles on growth, development and enzymatic activity of micromycetes (Sîrbu et al., 2015), bacteria (Santimano et al., 2013), biosynthetic potential and enzymatic activity of yeasts (Padroval et al., 2016) has demonstrated different effects depending on the nanoparticles structure and biotechnological object.

Inorganic compounds with nanodimensions possess high activity in low concentrations due to the large area of ratio the surface to volume and unique physical and chemical properties (Rai et al., 2009). Nanoparticles also demonstrate high stability in extreme conditions such as high temperature and pressure (Sawai, 2003). Some nanoparticles are non-toxic and contain essential mineral elements for human organism (Roselli et al., 2003). Recent studies offer perspective on the potential of ZnO nanoparticles utilization as supplement in order to increase active production of β-glucosidase - enzyme of *S. cerevisiae* with industrial value, as well as to increase production of baker's yeast (Ban et al., 2014).

Saccharomyces cerevisiae yeasts and their metabolites inclusive components of cellular wall are considered as safe materials by US Food and Drug Administration (FDA, 1997) and could be used legally as food ingredients and food supplements (Kwiatkowski et al., 2012). β-glucans obtained from yeasts serve as components of industrial bioproducts such as BetaRight® and WGP® (Biotheras, Inc.) proposed for application as ingredients of baking, drinks, yogurts, juice, chocolate and thickening agents for salad dressing, ice cream, mayonnaise, sauces and cheeses (Zechner et al, 2009).

The search of the new ways for development of microbial innovative biotechnologies has a great importance. Currently, the possibility to stimulate metabolic processes at yeasts by the

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application of nanoparticles is insufficiently studied. The research carried out on some yeast strains have demonstrated contradictory results. Given the fact, that products obtained from yeasts are inoffensive for human organism and perspective of nanomaterials utilization in different areas, the study of metal oxide nanoparticles influence on biosynthetic potential of *Saccharomyces cerevisiae* yeast strain are of scientific interest. The results of the following study can serve for elaboration of some efficient procedures for obtaining of biomass with predictable biochemical content.
Biochemical composition of yeasts could be regulated by the variation of cultivation conditions, as well as by the utilization of some stimulators. The adaptive cell response varied depending on strain. Accordingly, on the first stage of the study, the influence of alcohol that can be accumulated in large amount during industrial fermentation and deviated biosynthetic activity of yeasts is studied in this research. Further, experiments for monitoring cellular adaptive response at the cultivation in presence of ZnO nanoparticles in combination with alcohol are carried out to determinate the possibility for neutralization of toxic effects of alcohol on yeasts metabolism applied in high concentrations.

MATERIALS AND METHODS

Object of study. Yeast strain *Saccharomyces cerevisiae* CNMN-Y-20 from Collection of Yeasts Biotechnology Laboratory and the Collection of Nonpathogenic Microorganisms of the Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova producer was used in this study; the yeast strain is a β-glucans producer (Chiseliţa et al., 2010).
ZnO nanoparticles (NPs) with dimension of 30 nm, stabilized in polyvinylpyrrolidone (PVP) were offered by researchers of the Institute of Electronic Engineering and Nanotechnologies "D. Ghitu" of Academy of Sciences of Moldova (Guţul et al., 2014). The NPs concentrations used in the cultivation of yeasts were 5, 10 and 15 mg/L. Ethyl alcohol was added to culture medium in volumes of 2%, 5% and 10%. The sample without nanoparticles and alcohol served as control.
Culture medium and cultivation conditions. Fermentation medium YPD and beer wort were used for the inoculation and submerged cultivation of yeasts (Agiular et al., 2003). The submerged cultivation was effectuated in Erlenmeyer flasks with capacity 1 L on shaker with 200 rpm rotation speed at the temperature 25^0C, aeration rate 81.3...83.3 mg/L. The duration of cultivation was 120 hours. The volume of inoculated yeasts cells constituted 5% ($2x10^6$ cells/ml).
Research methods. Yeasts biomass was determined gravimetrically (Hong-Zhi et al., 2009). The carbohydrates content in the yeasts biomass was determined spectrophotometrically at 620 nm wavelength using antron reagent and D-glucose as standard (Dey et al., 1993) by using a PG T60 VIS spectrophotometer. The β-glucans content in the yeasts biomass was quantified gravimetrically as described (Thammakiti et al., 2004). Protein was determined by Lowry method (Lowry et al., 1951) using bovine serum albumin as standard. Catalase activity was determined by the method described in (Aebi, 1984, Efremova et al., 2012).
Statistical processing of results was carried out using statistical software kit 7. Statistical processing of obtained results was effectuated electronically with the calculation of the standard errors for the relative and average values, the differences between the experimental and control data were established using Student's t-test and P value.

RESULTS AND DISCSSIONS

The results demonstrate significant modifications of cellular components content at the cultivation of *Saccharomyces cerevisiae* CNMN-Y-20 strain using YPD medium supplemented with 2%, 5% and 10% alcohol. Thus, alcohol added in selected concentrations demonstrates beneficial effect on β-glucans biosynthesis, their content was increased by 10-13% compared to control (Figure 1). Even so, response reaction of yeasts to alcohol presence in 10% concentration is manifested by the significant decrease of biomass content to 1.55 g/L that is with 71% less than control. It is necessary to mention that protein content is decreased, also, in limits of 10-15% at the application of alcohol in 2, 5 and 10%

concentrations. The same inhibiting effect of alcohol is observed in the case of determination of catalase activity, antioxidant enzyme. However, catalase activity is increased at the use of 10% alcohol that served as toxicity index (Figure 1).

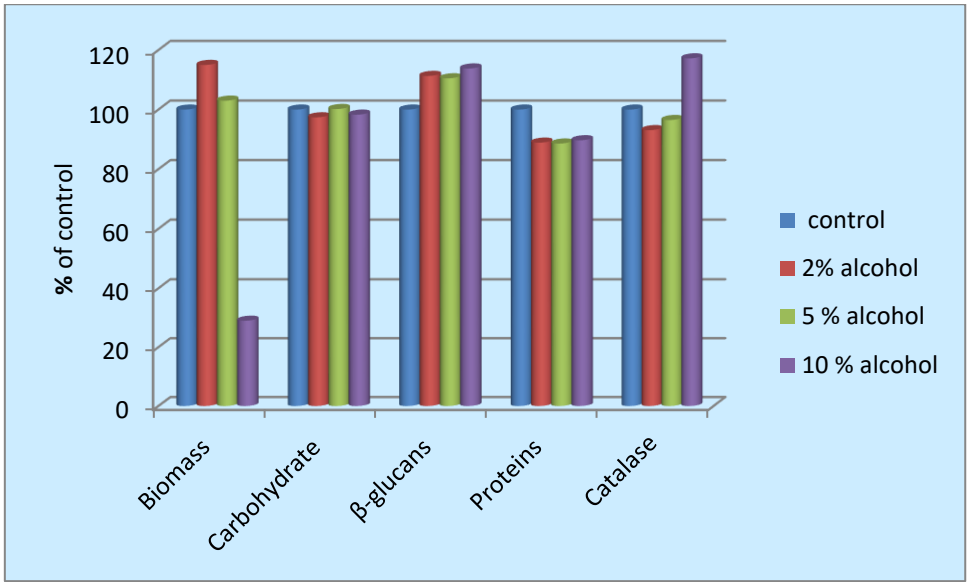

Figure 1. The degree of modification of the bioactive principles content at the cultivation of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain under the influence of alcohol, % of control.

Therefore, on the base of obtained experimental results it can be mentioned that the combination of 5% alcohol with 10 mg/L and 15 mg/L of ZnO nanoparticles contributed to decrease of cell multiplication, thus, biomass content after 120 hours of submerged cultivation on YPD medium was less with 14-18% compared to control (Figure 2). The quantitative determination of the proteins in *Saccharomyces cerevisiae* CNMN-Y-20 biomass reveals the fact that ZnO nanoparticles applied in combination with 2 % alcohol do not modifies significantly the content of these. The protein content is decreased with 4-7% comparative to control in experimental variants alcohol + ZnO nanoparticles.

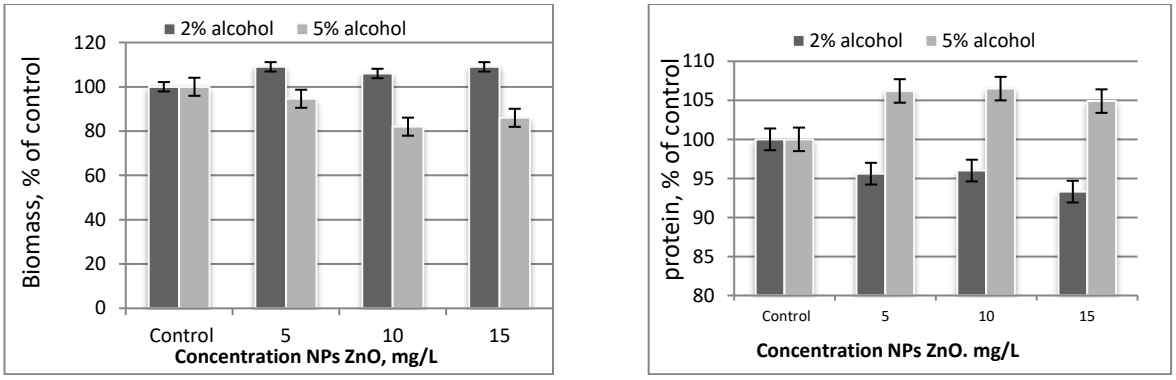

Figure 2. Effects of 30 nm ZnO nanoparticles in combination with alcohol etilic on biomass and protein content at *Saccharomyces cerevisiae* CNMN-Y-20

At the same time, results of the influence of ZnO nanoaprticles added in combination with higher alcohol concentration of nutrient substrate (5% alcohol) indicates another effect on protein content compared to combination of 2% alcohol with ZnO nanoparticles in 5, 10, 15 mg/L. Thus, increasing of the concentration of alcohol up to 5% in combination with nanoparticles contributes to the insignificant increase of protein content with 4-5% compared to control (Figure 2). Therefore, the analysis and interpretation of experimental results regarding the protein content at presence of alcohol and ZnO nanoparticles has demonstrates that ZnO nanoparticles partly neutralize the harmful effect of alcohol on the

process of proteins biosynthesis. In this case, the effect of nanoparticles can be explained by the possible involvement of Zn ions in biosynthetic processes and processes of regeneration of cell membrane via enzymes catalysing these processes.

Concerning the carbohydrates and β-glucans content in yeasts biomass, it was determined that combination alcohol - ZnO nanoparticles caused activation of biosynthetic processes. The obtained results indicate the cumulative positive effect of these two factors of cultivation on studied parameters. The content of carbohydrates and β-glucans increases depending on concentration with 10.7-16.6% and 12.1-19.9% compared to control, respectively (Figure 3). The maximal stimulatory effect relative to β-glucans content is detected at the use of combination of 2% alcohol+5 mg/L ZnO nanoparticles.

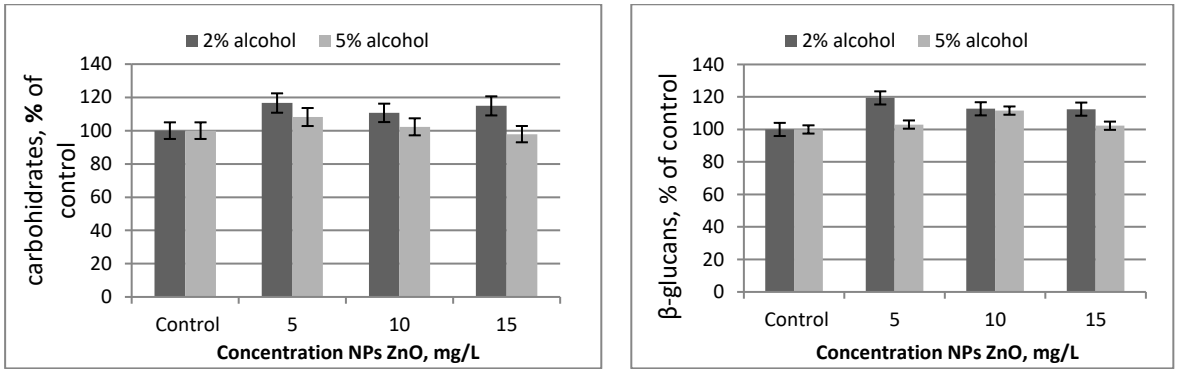

Figure 3. Effects of 30 nm ZnO nanoparticles în combination with alcohol on carbohydrates and β-glucans content at *Saccharomyces cerevisiae* CNMN-Y-20

Antioxidant enzyme activity levels have a great importance for toxicity level determination. The cumulative results were obtained at the determination of catalase activity at the cultivation of yeasts in presence of ZnO nanoparticles in combination with alcohol. Catalase activity varied insignificantly depending on concentration of nanoparticles used at cultivation of yeast strain (Figure 4). Thus, catalase activity increases with 10-16% compared to control at the highest concentration of nanoparticles - 15 mg/L.

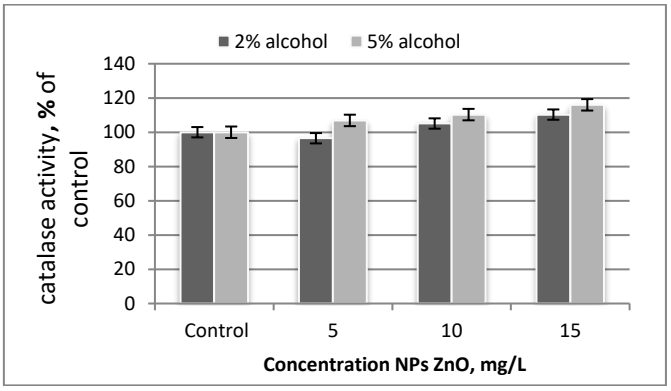

Figure 4. Catalase activity in *Saccharomyces cerevisiae* CNMN-Y-20 biomass at the cultivation in presence of 30mn ZnO nanoparticles in combination with alcohol

Taking into consideration that administration of ZnO nanoparticles in combination with alcohol has benefited action on β-glucans values at yeast strain, the comparative analysis of growth rate of cellular biomass and β-glucans content was carried out. The results demonstrated that specific growth rate of yeasts is maximal (6.02-6.16 g/L dry biomass) at the cultivation in presence of ZnO nanoparticles compared to other experimental samples. Minimal growth rate (1.55 g/L dry biomass) was determined at the cultivation in presence of 10% alcohol (table 1). In the case of determination of β-glucans content, the

highest values (21.25% - 22.55%) are obtained at the cultivation on substrate supplemented with 2% alcohol and ZnO nanoparticles. while values of β-glucans in control sample constituted 18.85. The level of the β-glucans production in experimental variants varies from 0.32 g/L to 1.32 g/L with the maximal values at 2% alcohol and 5 mg/L ZnO nanoparticles in culture medium (Table 1).

Table 1. The rate of biomass and β-glucans values at *Saccharomyces cerevisiae* CNMN-Y-20 at the cultivation on YPD medium in presence of ZnO nanoparticles and alcohol

Concentration of alcohol (%, v/v)	NPs ZnO concentration (mg/L)	Specific rate of β-glucans content (% dry.weight.)	Specific rate of dry biomass content, g/L	Content of β-glucans	
				g/L	% control
0	0	18.8±1.4	5.37±0.45	1.01	100
2	0	20.01±3.4	6.06±1.3	1.21	119.8
5	0	20.86±5.5	5.52±0.9	1.15	113.8
10	0	21.0±1.6	1.55±0.3	0.32	31.7
0	5	20.52±1.0	6.02±0.08	1.23	121.7
0	10	19.14±0.17	**6.16±0.28**	1.18	116.8
0	15	18.78±109	6.02±0.18	1.13	111.0
2	5	**22.55±1.9**	5.88±1.2	**1.32**	**130.7**
2	10	21.28±1.9	5.81±1.5	1.24	122.7
2	15	21.25±2.4	5.74±1.3	1.21	119.8
5	5	19.5±1.9	5.08±2.4	0.99	98.0
5	10	21.08±2.5	4.4±2.1	0.92	91.1
5	15	19.32±2.3	4.65±2.3	0.89	88.1

Analysing the experiments results, a new process for the direct β-glucan synthesis is recommended, which consists in adding to the YPD nutrient medium 5 mg/L of ZnO nanoparticles in combination with the alcohol in a 2% volume. Application of the process allows to obtain 1.32 g/L β-glucans, which are with 30.7% more than control.

CONCLUSIONS

1. Alcohol in 2%, 5% and 10% concentrations added to the cultivation medium of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain stimulates carbohydrates biosynthesis, including β-glucans and, at the same time, inhibits protein synthesis. Adaptive response of yeast strain to the presence of alcohol in 10% concentration is manifested by the decrease of cellular biomass with 71%, compared to control.
2. ZnO nanoparticles in presence of 2% and 5% alcohol concentrations stimulate processes of carbohydrates and β-glucans biosynthesis, their content in *Saccharomyces cerevisiae* CNMN-Y-20 biomass being with 16.6% and, respectively, 19.9% more than control, but did not provide sufficient protection for protein biosynthesis.
3. The maximal increase of β-glucans content is obtained at the cultivation of *Saccharomyces cerevisiae* CNMN-Y-20 in presence of 5 mg/L ZnO nanoparticles and 2% alcohol. Biomass production is considerable in samples with addition of nanoparticles in 5-15 mg/L concentration to culture medium.
4. The supplementation of ZnO nanoparticles in combination with alcohol to the substrate of *Saccharomyces cerevisiae* CNMN-Y-20 allowed to propose a procedure for activation of process of β-glucans biosynthesis to obtain β-glucans with 30.7% more than control.

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