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THE EVALUATION OF NANOPARTICLES ZnO AND TiO_2 EFFECTS ON *SACCHAROMYCES CEREVISIAE* CNMN-Y-20 YEAST STRAIN

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Abstract: This paper investigates the action of nanoparticles ZnO (10 nm) and TiO_2 (30 nm) on growth of *Saccharomyces cerevisiae* CNMN-Y-20 yeast. Nanoparticles in concentration of 0,5; 1,0 and 5,0 mg/L in YPD medium did not modify significantly cell proliferation, biomass production, the carbohydrate content and the content of β-glucans at *Saccharomyces cerevisiae* CNMN-Y-20. Nanoparticles ZnO and TiO_2 contributed to the decrease in protein content, which demonstrated the appearance of the alterations of yeast cell membranes.

Keywords: *Saccharomyces cerevisiae*, nanoparticles, multiplication, carbohydrates, β-glucans, proteins.

INTRODUCTION

The application of inorganic nanoparticles at the cultivation of microorganisms presents recent field of investigation reffering to the nanobiotechnology. According to the hypothesis, nanoparticles (NPs) may influence on the development of microorganisms (Rai et al, 2011). Results of the study of influence of various nanoparticles (oxide of Au, Ag, Ti, Si, Zn) on microbial cells, which may have stimulating or inhibiting effect on growth of microorganisms in dependence on composition or concentration, are exposed in some scientific papers (Ban et al, 2014; El-Diasty et al, 2013). The requirements to food industry nanotechnologies are listed in publications of Food Science Department, University of Massachusetts (Weiss et al, 2006).

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Biotechnological perspectives referring to the application of nanoparticles ZnO in various fields are mentioned in some publications (Espita et al, 2012; Vaseem et al, 2010) relating to the role of these in cosmetic products for nutritional toxicoinfection prevention due to the antimicrobial activity of different nanoparticles, etc. It is important to mention that nanoparticles ZnO in concentrations more than 50 ppm (50 mg/L) were characterized by toxic effect for *Saccharomyces cerevisiae* (Garcia-Saucedo, 2010).

Another type of nanoparticles with regulatory effect is titanium dioxide nanoparticles. Among other antimicrobial agents, TiO_2-NPs are of great scientific interest because of their high stability, are non toxic and safe for environment, low cost price, possess biological activity, etc.

Possible mechanisms of influence of nanoparticles TiO_2 at cellular level have been had investigated by more researchers that elucidated some processes that take place in the case of the application of nanoparticles (El-Said et, 2014; Minju et al, 2013). According to experimental literature data, the minimum inhibitory concentration (MIC) of nanoparticles is varied and depends on investigated microorganism. For example, MIC of nanoparticles TiO_2 for *Escherichia coli* and *Candida albicans* constituted 9,7 $\mu g.ml^{-1}$, for *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* MIC was 19,0-19,5 $\mu g.ml^{-1}$ (Piskin et al., 2013).

Thus, modifications of physiological state of yeast cells and response to the influence of external factors are closely interrelated with structure and dynamics of cell wall components. According to some researchers (Chang et al, 2012), the mechanism of influence of nanoparticles CuO and ZnO on the microbial cell is complex and leads to modifications as in cellular membrane, as also in cytoplasm. Given the fact that the composition of yeast biomass could be changed significantly by the utilization of culture medium, is important to establish factors that can improve biotehnological properties of microorganism - producer of bioactive substances.

The above mentioned emphasized the importance to evaluate the effects of ZnO şi TiO_2 nanoparticles on *Saccharomyces cerevisiae* CNMN-Y-20 producer strain of β-glucans and to assess the perspective of application of these in biotechnology of yeast cultivation.

MATERIALS AND METHODS

Research object. As an object of research served *Saccharomyces cerevisiae* strain CNMN-Y-20, producer of β-glucans, preserved in the Laboratory Yeasts Biotechnology and Collection of Nonpathogenic Microorganisms (Chiselita et al, 2010).

ZnO and TiO_2 nanoparticles with dimensions of 10 and 30 nm, polyvinylpyrrolidone (PVP) stabilized used for investigation were

generously provided by the researchers of the Institute of Electronic Engineering and Nanotechnology of Academy of Sciences of Moldova (Gutul et al., 2014). Nanoparticles were added as emulsion in the concentrations of 0,5; 1,0; 5,0 mg/L YPD medium to the yeast culture at the moment of seed material inoculation. The variant without nanoparticles application served as reference sample.

Media and culture conditions. YPD nutritive medium was used for inoculation and cultivation (Aguilar-Uscanga et al, 2003). The submerged cultivation was carried out in depth capacity 1 liter Erlenmeyer flasks on shaker (200 rpm.) at a temperature of +25ºC, aeration rate 80,0...83,0 mg/L, the duration of cultivation 120 hours. Yeast cells, in an amount of 5%, $2x10^6$ cells/ml were inoculated on the liquid medium.

Methods. The amount of cells developed at liquid medium was determined spectrophotometrically according to following method (Mitchell et al, 2004). Cell biomass was determined gravimetrically (Liu et al, 2009). The content of total carbohydrates was determined using PGT60 VIS spectrophotometer at wavelength of 620 nm with the utilization of antron reagent and D-glucose as standard (Dey et al, 1993). The content of β-glucans in the yeast biomass was determined gravimetrically as described (Thammakiti et al, 2004). Protein was determined by the Lowry method (Lowry et al, 1951) using bovine serum albumin as standard.

The oxygen content was measured by portable oximeter – Oxi-315i/SET 2B10-0011. The values of the average pH of cultivation were determined with pH-316i MeBketten WTW, Germany.

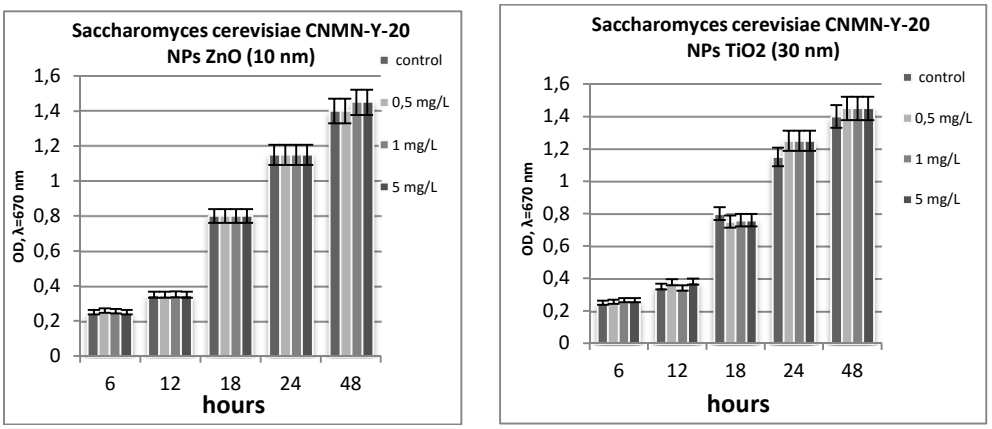
Statistical processing of obtained results was effectuated with set programs Statistics 7, using P - value $\leq 0,05$.

RESULTS AND DISCUSSIONS

Metal-based nanoparticles are various and have different shapes and sizes. Due to the sizes that are less than 100 nm and unique chemical and physical properties, nanoparticles may induce significant modifications in different biological systems. The graphic representation of the structure of some nanoparticles is reflected in publications of Citlali Garcia Saucedo (2010), which has mentioned that the effect on *Saccharomyces cerevisiae* was in dependence on nanostructure. So from that perspective, the most important aim of present research was the evaluation of effects of two types of nanoparticles – zinc oxide and titanium oxide on growth and productivity of *Saccharomyces cerevisiae* CNMN-Y-20.

To appreciate the rhythm of yeast growth, nanoparticles have been added in YPD culture medium in the concentrations of 0,5; 1,0 and 5,0 mg/L. The process of cells reproduction was monitored for 48 hours of submerged

cultivation, the period that includes all the phases of the process of multiplication of yeast cells: lag, log, stationary and cell death.
Experimental results of influence of ZnO and TiO_2 nanoparticles on cell reproduction are presented below in figure 1 that demonstrates that optical density determined at wavelength 670 nm not differ from the reference sample. The processes that take place in each of the stages of yeast growth correspond to classical schemes.

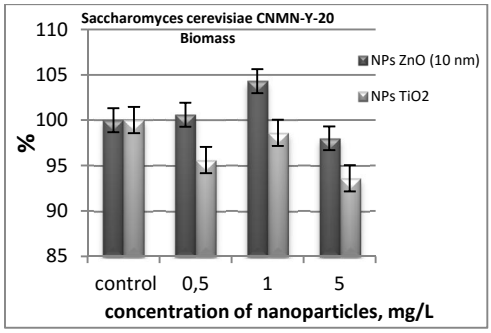


a) b)

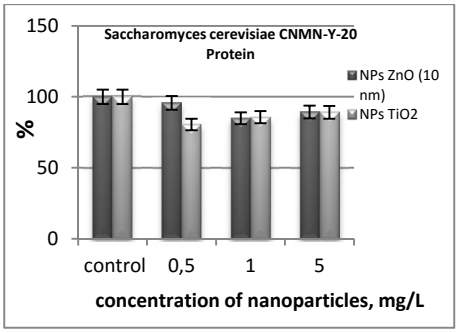
Figure 1. Values of optical density (OD) of yeast cells *Saccharomyces cerevisiae* CNMN-Y-20 in the presence NPs ZnO (a) and NPs TiO_2 (b)

It is known, that productivity indices of culture might be changed in modified physicochemical conditions. So, on that basis, the potential of cell biomass, proteins, carbohydrates, β-glucans production – indices that characterize the influence of nanoparticles on culture growth has been studied.
The study regarding the content of dry biomass obtained after 120 hours of submerged cultivation has revealed that *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain was resistant to ZnO nanoparticles. The concentration of 1,0 mg/L induced an insignificant accumulation (with 4,3 %) of biomass quantity. The analysis of the influence of TiO_2 nanoparticles contributed to the decrease with 3-6 % of biomass content compared with the indices of control (Figure 2).
An important indicator used to characterize metabolic pathways at yeasts exposed to the action of different cultivation factors is protein content. The comparative analysis of protein content accumulated at *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain has demonstrated a trend of decrease in comparision with control (Figure 2). The rate of decrease of protein content depends on chemical composition and concentration of nanoparticles. In the case of the concentrations of 1,0 - 5,0 mg/L of ZnO nanoparticles, the protein

content decreased with 10-15%, respectively. The experimental results regarding protein content in the case of utilization of TiO_2 nanoparticles have demonstrated values under control (decrease with 19,5%) for the concentration of 0,5 mg/L.



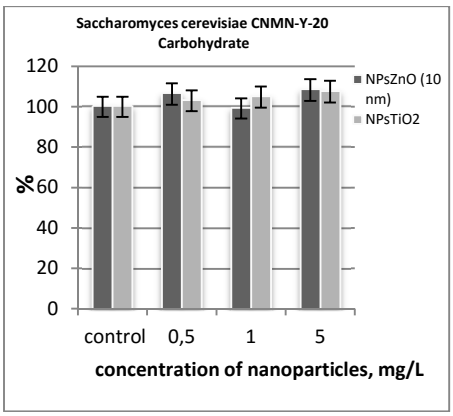
a) Biomass content



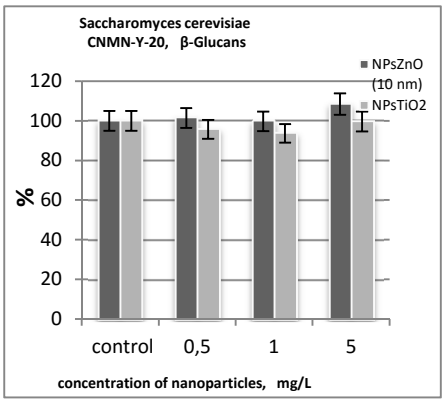
b) Protein content

Figure 2. Effect of nanoparticles ZnO (10 nm) and TiO_2 on biomass content (a) and protein content (b) at *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain

Then, a similar study has been carried out by the determination of the content of carbohydrates in yeast biomass with the utilization of ZnO and TiO_2 nanoparticles. The statistical analysis of obtained results has demonstrated different values of carbohydrates quantity that varied in dependence on concentrations of applied nanoparticles in interval 28,7 -31,3 % of dry biomass that comparatively corresponded to value of control which constitued 28,9% (Figure 3).



a) Carbohydrate content



b) β-glucans content

Figure 3. Effects of ZnO and TiO_2 nanoparticles on carbohydrates accumulation(a) and β-glucans (b) content at *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain

The determination of componence of polysaccharide, β-glucans, in yeast biomass at the cultivation in the presence of ZnO and TiO_2 has demonstrated insignificant modifications compared to control. The values of β-glucans content in experimental samples with the application of ZnO nanoparticles varied in interval 17,48 – 19,0% per dry biomass, while the amount of β-glucans constituted 17,53%. An insignificant increase of β-glucans content was revealed at the utilization of ZnO nanoparticles in concentration of 5,0 mg/L. The content of β-glucans at the cultivation of studied yeast strain on nutritive medium supplemented with TiO_2 nanoparticles constituted from 16,42% to 17,47% per dry biomass, that was with 5-6% lower than control (Figure 3). According to the presented figures there is no significant difference.

CONCLUSIONS

Thus, summarizing the experimental results reffering to the influence of nanoparticles on *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain, it can be mentioned that effectuated investigations did not establish any significant stimulatory effect of nanoparticles ZnO (10 nm) and TiO_2 (30 nm) in concentrations of 0,5; 1,0 şi 5,0 mg/L YPD medium on cell reproduction, biomass production, carbohydrates and β-glucans content. It has been revealed that nanoparticles contributed to the decrease in protein content. The results confirm the necessity to create evaluation models of potential dangers of use nanoparticles.

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