THE EFFECT OF Fe_3O_4 NANOPARTICLES ON BIOPRODUCTION PARAMETERS OF *Rhodotorula gracilis* CNMN-Y-30 YEAST STRAIN WITH HIGH BIOTECHNOLOGICAL POTENTIAL

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Abstract. The paper provides new data about the effect of Fe_3O_4 nanoparticles with dimensions of 10 nm and 30 nm on bioproduction parameters of *Rhodotorula gracilis* CNMN-Y-30 strain. It has been established that size of nanoparticles and their concentrations did not change cells prolificacy and the content of cell biomass. It was found that the protein content was higher in yeast biomass up cultivation in the presence of iron oxide nanoparticles with dimensions of 30 nm, particularly at low concentrations of 0.5 and 1.0 mg / L, compared to the 10 nm size nanoparticles.

Keywords: *Rhodotorula gracilis*; nanoparticles; multiplication; carbohydrates; proteins.

INTRODUCTION

Nanoparticles usually ranging in dimension from 1-100 nanometers (nm) have properties unique from their bulk equivalent. With the decrease in the dimensions of the materials to the atomic level, their properties change. The nanoparticles possess unique physico-chemical, optical and biological properties which can be manipulated suitably for required applications. Nanotechnology is rapidly growing by producing nanoparticles (NPs) that have novel and size-related physico-chemical properties. The novel properties of nanoparticles have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices [11, 24].

A type of nanoparticles with extensive applicability is represented by iron oxide nanoparticles, how it would be: magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), MFe_2O_4 (M = Mn, Mg, Ni, Co, Zn, etc.). The utilization of these forms for biomedical research , for example, for magnetic resonance imaging (MRI) as contrast agents, for marking tumor cells, for treatment of magnetic hyperthermia, for magnetic transport and targeting of medical substances led to significant improvement of medical technologies [3, 25]. The previous studies regarding the toxicity of irone oxides and solutions based on metal oxides have demonstrated their reduced toxicity [2, 4, 9, 22].

According to some researchers, the influence of Fe_3O_4(I), Fe_3O_4(II), and Fe(I) nanoparticles on protozoa cells was studied. Analysis of concentration effects revealed that nanoparticles of Fe (I) have stimulated the maximum toxic effect on protozoa cells which was observed at concentrations up to 4 x 10^{-5} M. The number of surviving cells was gradually increased (from 40 to 95%) in all other dilutions up to 6 x 10^{-6} M. Toxic effect of iron oxides also was established in the case of concentrations up to 9 x 10^{-5} M [10, 12, 14, 27]. Using well diffusion method antibacterial activity of Fe_3O_4 nanoparticles was tested against gram-positive and gram-negative *Staphylococus aureus, Xanthomonas, Escherichia coli and Proteus vulgaris*. Fe_3O_4 nanoparticles exhibited strong antibacterial activity against bacterial species [21]. The study of the of effect Fe_3O_4 on *Saccharomyces cerevisiae* yeast cells has demonstrated the reduced toxicity of nanoparticles used in concentration of 1000 mg/L [20]. Nanoparticles have been hypothesized to influence microbial production, growth and survival. Previous reports evidenced the effect of different NPs on the growth and secondary metabolite production in various microorganisms [13, 28].

In that regard, the aim of present study was to determine the effect of Fe_3O_4 nanoparticles on bioproduction parameters of *Rhodotorula gracilis* yeast strain which possess high biotechnological potential.

MATERIALS AND METHODS

Objects of study. Pigmented yeast *Rhodotorula gracilis* - CNMN-Y-30 was selected for the research. The strain is preserved in the collection of Yeasts Biotechnology Laboratory and in the Collection of Nonpathogenic Microorganisms of within Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova.

Nanomaterials. In the experiments Fe_3O_4 nanoparticles (10 and 30 nm) were used, the suspension was prepared according to the method specified Oterro-Gonzalez, et al. [20] made available for us with great kindness by researchers of the Institute of Electronic Engineering and Nanotechnologies “D. Ghitu”. The concentrations of nanoparticles used in experiments to cultivate yeasts constituted 0.5; 1,0; 5.0; 10 to 15 mg/L. The sonicated suspension of nanoparticles in volume that corresponds to studied concentration was added to the nutritive medium simultaneously with inoculum.

The variant without application of nanoparticles was used as control sample. Nanoparticles Fe_3O_4 or other sources of iron have not been added to the control sample.

Culture Media. For inoculation and submerged cultivation of yeasts were used fermentation media specific to strains in YPD study and wort malt [1].

Submerged cultivation was carried out in Erlenmeyer flasks with a capacity of 1.0 L, the rotating speed of the stirrer 200 rpm, at 25^0C, the degree of aeration 80.0...83.0 mg/L, the length of submerged cultivation 120 hours. Broth medium was seeded in an amount of 5% with the inoculum 2 x 10^6 cells/mL [18, 19]. The oxygen content was measured with the portable oximeter – Oxi-315i/SET 2B10-0011. The pH values of the cultivation environment were measured using the pH-316i MeB ketten WTW, Germany.

Methods of achieving research. The number of cells grown on liquid medium was determined spectrophotometrically according to known methods [19]. Yeasts Biomass was determined gravimetrically [15]. Total carbohydrates in the biomass of yeast were determined at the spectrophotometer PG T60 VIS Spectrophotometer at wavelength 620 nm using Anthrone reagent and D-glucose as standard [8]. Protein was determined spectrophotometrically according to the method of Lowry [16], using crystalline albumin from bovine serum as standard.

Statistical processing of results was done using statistical software kit 7 veracity compared to the control $p \leq 0.05$.

RESULTS

This study aim was to determine the influence of nanoparticles on some parameters of pigmented yeast strain with high biotechnology importance. This section presents the results of the influence of with two sizes (10 and 30 nm) Fe_3O_4 nanoparticles on cells proliferation, production of biomass, carbohydrate and protein content.

The nanoparticles were added to yeast culture as a solution in the concentration of 0.5, 1.0, 5.0, 10 and 15 mg/L to YPD medium at the time of inoculation with the seed material. Prolificacy of the culture was determined after 6, 24 and 48 hours of cultivation in depth. At the end of the life cycle, after 120 hours of cultivation, the yeast biomass was collected by centrifugation and subjected to biochemical analysis. The results obtained in establishing cell proliferation has demonstrated that in those experiences under the influence of nanoparticles with dimensions of 10 nm after 24 hours of submerged cultivation, prolificacy was decreased by 17-25% compared to control, but after 48 hours was recorded an activation of cell multiplication, which allowed to obtain an increasing by 12-18% (Fig. 1). The experimental results of the use Fe_3O_4 nanoparticles use with the size larger than 30 nm demonstrated some deviations from the control, especially in the first 6 hours of cultivation in the presence of concentrations of 15 mg/L, which then lines up to the prolificacy of control strain.

The biomass production is one of the most important indicators of medium components action on bacterial cells. In most of the cases, the productivity increasing is the result of an stimulating effect, at the same time, the decrease of biomass content is adaptive response to unfavorable condition. Analysis of the results of experiences for determining the effects of nanoparticles on biomass content revealed a relative stability for both dimensions of Fe_3O_4. Variations in the content of the biomass were established at the 0.5 and 1.0 mg/L to 10 nm nanoparticles, which increases slightly (by 13.3 to 14.8%) the cell biomass accumulation (Fig. 2).

Carbohydrates are important structural components of the yeast cell wall and present the main energy source for cellular metabolism. Against the insignificant modification of productivity, the reduction of carbohydrates biosynthesis can be qualified like the consequence of toxic action of

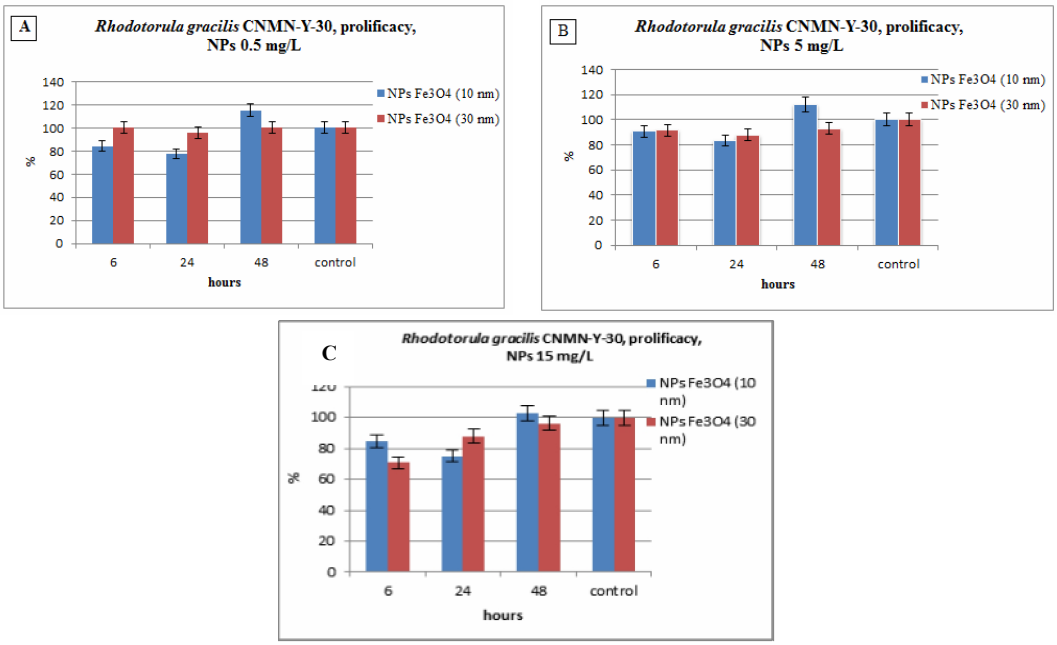

Figure 1. Prolificacy of *Rhodotorula gracilis* CNMN-Y-30, cultivated in the presence of Fe_3O_4 nanoparticles with different size - concentrations 0.5 (**A**); 5 (**B**) and 15 mg/L (**C**)

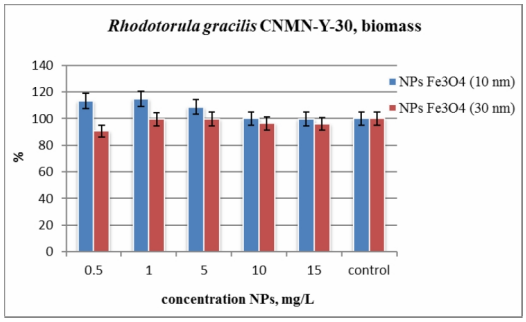

Figure 2. The content of the biomass *Rhodotorula gracilis* CNMN-Y-30, cultivated in the presence of Fe_3O_4 nanoparticles with different sizes and concentration

nanoparticles. In experimental variants of *Rhodotorula gracilis* CNMN-Y-30 strain cultivation, under the influence of Fe_3O_4 nanoparticles in the concentration range of 0.5-1.0, 5.0, 10.0 and 15.0 mg/L, results revealed the different values of the determined quantity of carbohydrates in the cellular biomass, values that are consistent with nanoparticles size. For example, an significant increase of the carbohydrates content in the yeast biomass cultivated in the presence of nanoparticles with sizes of 30 nm was established, values exceeded the control with 13-21.3%. In the experimental variants using Fe_3O_4 nanoparticles 10 nm, the results demonstrated lower values of the carbohydrates content compared to the control sample, especially under the influence of the concentration of 15 mg/L (Fig. 3). Thus, in the carried out researches it was demonstrated that, depending on the concentration and size, Fe_3O_4 nanoparticles can serve as regulators of carbohydrate biosynthesis.

The dependence between concentration of used for the studied nanoparticles and carbohydrates values is expressed by coefficient of determination, denoted R^2 = 0,67 for nanoparticles with size of 10 nm. In this case, the correlation analysis has demonstrated that nanoparticles may affect the carbohydrate biosynthesis. It was determined that coefficient of determination R^2 =0 for nanoparticles with size of 30 nm demonstrated the absence of the dependence between the values of studied parameters (Fig. 3).

It is known that iron is an essential metal in cellular respiration and maintains the alkalinity of the body. The most important role of iron in the body is binding and transport of atmospheric oxygen. The ionized form of the iron is toxic, which is why the iron is always associated with haemin of proteins or linked to specific storage or transport proteins such as transferrin, ferritin and haemosiderin. In this context, the current research on the effect of magnetic iron oxide nanoparticles in the formation of protein in yeast is actual, some results that would allow some application predictions in biomedicine of bioproducts derived from yeast.

In present experiences, Fe_3O_4 nanoparticles with dimensions of 30 nm occurred as protein stimulator in all of the tested concentrations. The increase of protein content in yeast biomass at the cultivation on YPD medium with the addition of these nanoparticles was 14.1 to 23.8% compared to the reference sample. The process of yeast cultivation on medium with the addition of Fe_3O_4 nanoparticles with dimensions of 10 nm did not have the same reaction. Moreover, the utilization of concentration of 15 mg/L of nanocompounds has reduced the protein content by 28.8% compared to the control (Fig. 4).

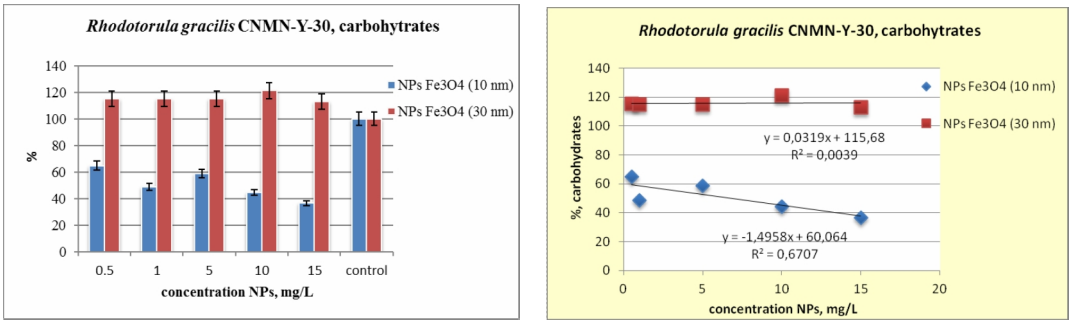

Figure 3. The content of carbohydrates in *Rhodotorula gracilis* CNMN-Y-30 biomass, cultivated in the presence of Fe_3O_4 nanoparticles with different sizes and concentration

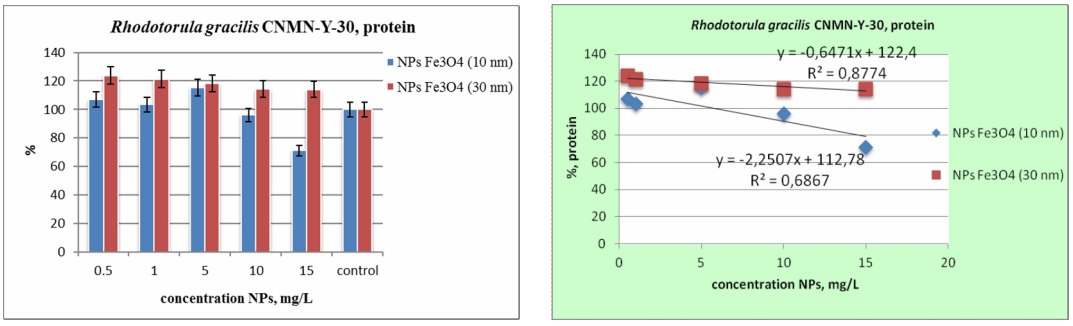

Figure 4. The protein content in *Rhodotorula gracilis* CNMN-Y-30 biomass, cultivated in the presence of Fe_3O_4 nanoparticles with different sizes and concentration

The correlation analysis of protein content and concentration of used nanoparticles was carried out to establish the determinant role of nanoparticles. In the case of Fe_3O_4 nanoparticles with size of 10 nm application, the large correlation between concentration of nanoparticles and protein values has been revealed, coefficient of determination R^2 =0,68. Very strong correlation (R^2 = 0,87) between studied parameters was established at the utilization of nanoparticles with size of 30 nm. Therefore, protein content of *Rhodotorula gracilis* CNMN-Y-30 was affected by the concentration of used nanoparticles.

Thus, the present experimental study brings argumentation in the favour of utilization of nanoparticles Fe_3O_4 as an important factor for the biotechnology of yeasts cultivation. Therefore, investigations are opportune for the elaboration of new procedures of utilization of nanoparticles to increase bioproductive potential of some yeast strains.

DISCUSSION

This paper presents the results of a reserch on the influence of the nanoparticle of Fe_3O_4. with the size of 10 nm and 30 nm of pigmented yeast *Rhodotorula gracilis* CNMN-Y-30.

The study of Fe_3O_4 nanoparticles influence on the culture of *Rhodotorula gracilis* CNMN-Y-30 demonstrated that the effect depends on their size and concentration.These results have been confirmed by other researchers which have established that the action of iron oxide nanoparticles was specific and depending on type of cell. It is noted by authors that nanoparticles have no essential impact on viability of some cells [5]. It was experimentally revealed that degree of toxicity of nanoparticles depends on their concentration and duration of exposition to cells [7]. Shahzeidi et al., 2015 [26] has demonstrated antibacterial effect Fe_3O_4 nanoparticles at *Pseudomonas aeruginosa, Escherichia coli și Staphylococcus aureus.* It was established that size and concentration of Fe_3O_4 nanoparticles might affect antibacterial properties of studied starins. The phenomena observed, in most of the cases is explained by mechanisms of action of metallic nanoparticles on cells, the most important are those of oxidative stress, non-homeostase effect [6, 23].

In experimental variants was established an insignificant stimulation of cell prolificacy under the influence of the nanoparticles with the size of 10 nm, an effect that was not observed in the variants with nanoparticles of 30 nm.

Evaluation of the composition of yeast biomass in experimental variants with nanoparticles was effectuated. Also, in the case of determining of cells prolificacy it was established that this one practically did not change depending on Fe_3O_4 nanoparticles concentrations and dimensions.

Nanoparticles of iron oxide with the dimensions of 30 nm, in concentrations of 0.5-15 mg/L assures the moderate increase of the amounts of carbohydrates in biomass of *Rhodotorula gracilis* CNMN-Y-30 and contributes to significant decrease of this index at the cultivation of selected yeast strain in the presence of 10 nm nanoparticles.

The degree of stimulation of the protein content was higher in the yeast biomass cultivated in the presence of iron oxide nanoparticles with a size of 30 nm, particularly at low concentrations of 0.5 and 1.0 mg/L, compared to nanoparticles with a size of 10 nm.

The synergistic obtained effect can be explained by the modification of the degree of permeability of cell membrane by nanoparticles. Other researchers have demonstrated that impact of nanoparticles on cell integrity depends on the structure and size of nanoparticles [17, 20]. Further, it is important to study the influence of Fe_3O_4 nanoparticles on biosynthesis of other cell components, such as carotenoids.

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