

# ESTIMATION OF THE EFFECTS OF CHITOSAN-IRON NANOCOMPOSITES DEVELOPED BY DIFFERENT PROCESSES ON *R. GRACILIS* CNMN-Y-30 YEAST

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**Abstract.** The paper provides information on the estimation of the effects of iron chitosan nanocomposites, elaborated by different procedures on pigmented yeast *Rhodotorula gracilis* CNMN-Y-30. It was found that the initial amount of chitosan, the concentration of Fe<sub>3</sub>O<sub>4</sub> nanoparticles and the volume of nanocomposite used for growing yeasts are the main factors that influence the efficiency of chitosan-iron nanocomposites. Microbiological indices adequately reflect the effects of chitosan-iron nanocomposites in the process of evaluating the action of nanocomposites obtained by different processes on the representative yeast *R. gracilis* CNMN-Y-30 and it is recommended to test the degree of influence of the nanocomposite. This information can be used by specialists in the food industry, microbiology, medicine, cosmetology, environmental protection, etc., where nanocomposites have applications.

## INTRODUCTION

Iron oxide nanoparticles (Fe<sub>3</sub>O<sub>4</sub>) offer attractive possibilities for applications in biotechnology, biomedicine, cosmetology, pharmacology, nutrition, etc. (Blaney, 2007; Shahzeidi et al., 2015). However, high toxicity and low stability produce impediments for use (Puja et al., 2015; Sarlo et al., 2009; Usatii, et al., 2017). Due to their small size and large surface / volume ratio, the nanoparticles tend to form agglomerations; they also have the ability to easily oxidize in air. It is therefore necessary to modify the surface to stabilize the Fe<sub>3</sub>O<sub>4</sub> nanoparticles and to avoid oxidation processes. These shortcomings can be reduced by combining nanoparticles with different polymers, in particular polysaccharides (Silva et al., 2013; Sonaje et al., 2011; Zlotski et al., 2017). Polysaccharides are non-toxic, biodegradable, and biocompatible with the environment. A new approach for broadening the spectrum of use of iron oxide nanoparticles presents their coverage with chitosan. Chitosan is of interest as a biopolymer to efficiently stabilize metal oxide nanoparticles, offering increased biocompatibility and chemical functionality (Dias et al., 2011; Vikele et al., 2017).

The modified surface of nanoparticles in combination with polysaccharides may initiate different effects of the nanocomposite. Generally, chitosan-iron nanocomposites are required in applications in the transport of anticancer drugs (doxorubicin, 5-fluorouracil and leucovorin (Kevin et al., 2001, Tan et al., 2009). They may also participate in the release and administration of cDNA against hepatitis B through the nasal mucosa (Khatri, 2008); protein delivery (Sonaje, 2011), insulin administration (Finotelli et al., 2010), release of polyphenolic antioxidants reduce the cytotoxic effect on living cells (Zheng, 2011) and have antimicrobial effect (Qi, 2004). In biotechnology, the iron chitosan nanocomposite can be applied to immobilization of penicillin G acylase (Xiao-Min Ling, 2016). In remediation of the environment it can participate in the cleaning of the water and soil of persistent organic pollutants (Huang et al., 2015; Tang et al., 2013; Gutierrez et al., 2017).

However, recent advances in investigating the toxicity of nanocomposites have proposed both safety and risk at the same time. However, it was still essential to consider the potential danger posed by nanocomposites with different sizes and concentrations on biological objects (Bui et al., 2017). The influence of iron chitosan nanocomposites can be assessed by means of *R. gracilis* pigmented yeasts, which can serve as a biotechnological object but also as a test model organism. Therefore, the assessment of the more acute and sub-acute toxicity of nanocomposites in vivo should be carried out to avoid potential hazards in the future (Wang et al., 2017).

Taking into account the above, the purpose of the research is to estimate the effects of chitosan-iron nanocomposites, elaborated by different procedures, on yeasts of the genus *Rhodotorula*, in the context of establishing the potential for use.

## MATERIALS AND METHODS

*Objects of research.* Pigmented yeast strain *Rhodotorula gracilis* CNMN-Y-30, producer of proteins and carotenoids, was selected for the research (Usatii et al., 2016). The strain is preserved in the collection of Yeasts Biotechnology Laboratory and in the Collection of Nonpathogenic Microorganisms of Institute of Microbiology and Biotechnology of Moldova.

*Nanomaterials.* In the experiments Fe<sub>3</sub>O<sub>4</sub> nanoparticles (NPs) 50-100 nm in the form of powder, surface area > 60 m<sup>2</sup>/g, density 4.8-5.1 g/ml at 25<sup>o</sup> C (Aldrich). The nanoparticle stock solution was prepared according to the method outlined by (Otero-Gonzalez et al., 2013). The NPs suspensions were prepared by the sonochemical method, stabilization was performed in chitosan polymer (Aldrich). Coating of NPs Fe<sub>3</sub>O<sub>4</sub> with chitosan was prepared according to the method specified (Mohammadi-Samani et al., 2013).

The experimental procedure I. To 25 mg chitosan was added 25 ml of 1% acetic acid and stirred for 10 minutes at 200 r. p.m. Subsequently, the neutral pH with NaOH is established, 1% of ethyl alcohol of 96% is added. It is stirred for 10 minutes at 200 r.p.m. In the chitosan solution add the Fe<sub>3</sub>O<sub>4</sub> nanoparticles (50-100 nm) at 50 mg/L and 70 mg/L and sonify for 10 minutes. During this process chitosan molecules are absorbed on the surface of metal nanoparticles.

The experimental procedure II. To 50 mg chitosan was added 25 ml of 1% acetic acid it is prepared according to the procedure indicated in procedure I.

Control procedure I. Chitosan-iron nanocomposite is prepared according to the method proposed by Mohammadi-Samani (2013). 20 mg chitosan is dissolved in 1M acetic acid solution with a final volume of 100 ml. Then, 70 mg of Fe<sub>3</sub>O<sub>4</sub> nanoparticles is added to the above solution. The mixture is stirred for 18 hours until, a homogeneous dark brown solution is obtained.

Control procedure II. Yeast strain is grown on YPD culture medium, without the introduction of chitosan-iron nanocomposites.

*Culture Media.* YPD (yeast-peptone-dextrose) fermentation medium and wort was used to obtain the seed material and to grow the yeasts *Rhodotorula gracilis* CNMN-Y-30 (Aguilar-Uscanga et al., 2013). Submerged cultivation will be carried in Erlenmeyer flasks 1.0 L, the rotating speed of the stirrer 200 rpm, at +25...27°C, the degree of aeration 80.0...83.0 mg/L, permanent lighting 2000 Lx, the time of cultivation 120 hours. Broth medium was seeded in an amount of 5% with the inoculum 2 x 10<sup>6</sup> cells/mL.

*Methods of achieving research.* The viability of yeast *Rhodotorula gracilis* CNMN-Y-30 was determined by the microbial counting method which consists in performing the serial dilutions of yeast suspension with subsequent, plating in agarized YPD medium and counting of total colonies. Manual counting was performed (Концевая et al., 2011). Productivity of yeasts biomass was determined gravimetrically (Hong-Zhi et al., 2009).

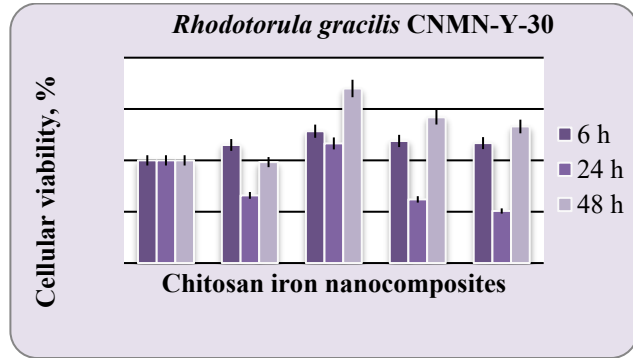
The data results of 3-5 repetitions obtained were expressed by calculating the mean, standard deviation and confidence interval for an average. All differences were considered statistically significant for P ≤ 0.05, compared to the control variant.

## RESULTS AND DISCUSSIONS

The preparation method plays an important role in obtaining nanocomposites adapted for practical applications. Within the experiences presented in this study, was evaluated the action of the chitosan-iron nanocomposite, applied in the cultivation environment in volumes of 2% and 5%, on the cell viability and biomass production in the yeast strain *R. gracilis* CNMN-Y-30.

As a result of the research, it was found that the chitosan-iron nanocomposites prepared according to the experimental procedures I and II, invoke different effects compared to the control procedure proposed by Mohammadi-Samani (2013). The quantification of cell viability in the yeast strain *R. gracilis* CNMN-Y-30 demonstrated the tendency to increase the number of cells only in the variant in which the nanocomposite prepared according to the experimental procedure I in 2% volume was added (fig. 1). Cellular viability increases by up to 16.5% compared to chitosan-iron nanocomposite prepared according to the Mohammadi-Samani control procedure (2013). At the same time, the nanocomposite prepared according to the experimental procedure I, but introduced into the culture medium for yeasts by 5% volume, leads to a statistically reliable reduction (up to 50% compared to the control procedure) of cell viability for

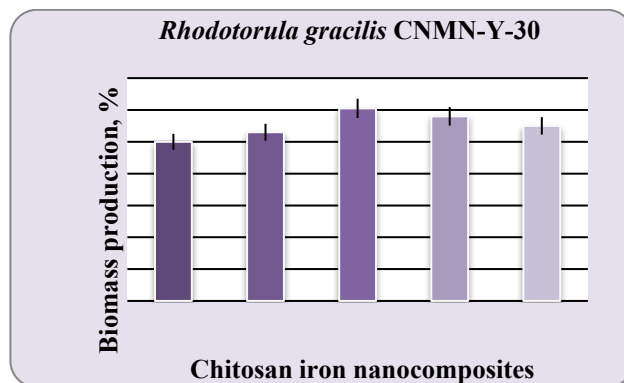
24 hours. Pronounced toxic effect on the viability of yeast cells, showed and nanocomposite prepared according to experimental procedure II used both in volume of 2% and 5%. After 6 and 24 hours of contact with the nanocomposite, the essential reduction of the number of viable cells (by 84-85% compared to the control procedure) is observed (fig. 1).



Legend: 1 - Control (YPD); 2 - Control procedure I; 3,4,5 - experimental procedure I, with the content of  $\text{Fe}_3\text{O}_4$  nanoparticles in concentration of 30, 50 and 70 mg/L, respectively.

Fig.1. Cellular viability of yeast strain *R. gracilis* CNMN-Y-30 under the action of chitosan-iron nanocomposites, obtained by different processes

The results of the evaluation of the influence of the chitosan-iron nanocomposites obtained by different processes on the production of biomass *R. gracilis* CNMN-Y-30 showed similar changes to those exposed for the viability of the cells. The resulting study shows that in the case of the application of the nanocomposite prepared according to the experimental procedure I, introduced in the culture medium for yeasts by volume of 2%, after 120 hours of cultivation, it initiates the increase of the cellular biomass quantity (fig. 2). The pronounced toxic effect on the accumulation of the yeast biomass exerted the nanocomposite prepared according to the experimental procedure II, which applied in volume of 2% and 5%, causes the essential reduction of the biomass quantity compared to the control process.



Legend: 1 - Control (YPD); 2 - Control procedure I; 3,4,5 - experimental procedure I, with the content of  $\text{Fe}_3\text{O}_4$  nanoparticles in concentration of 30, 50 and 70 mg/L, respectively.

Fig.2. Biomass production at the yeast strain *R. gracilis* CNMN-Y-30 under the action of chitosan-iron nanocomposites, obtained by different processes.

Thus, the results of the tests showed that the most efficient process for the formation of nanocomposites is the experimental procedure I, which highlighted new aspects regarding the viability and differentiated development of the yeast *R. gracilis* CNMN-Y-30 under the action of the chitosan-iron nanocomposite compared to the cultivation in common conditions. The results of the study contribute to the efficiency of the research on elucidation of the processes or mechanisms of action of the nanocomposite on the metabolism of the yeast cells.

## CONCLUSIONS

Generalizing the results obtained in this study it can be mentioned that the the initial quantity of chitosan, the concentration of metallic nanoparticles and the volume of nanocomposite used for the cultivation of yeasts are the main factors that influence the efficiency of the chitosan-iron nanocomposites.

The optimal variant for the preparation of chitosan - iron nanocomposites is the experimental procedure I to 25 mg chitosan was added 25 ml of 1% acetic. For the submerged cultivation of yeasts, the nanocomposite is added to the YPD culture medium in volume of 2%. The microbiological indices adequately reflect the effects of chitosan-iron nanocomposites in the process of evaluating the action of the nanocomposites obtained by different processes on the representative yeast *R. gracilis* CNMN-Y-30 and it is recommended to test the degree of influence of the nanocomposites on the yeasts.

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