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BIOLOGICAL EFFECT OF SOME METAL OXIDES NANOCOMPOSITES ON *SACCHAROMYCES CEREVISIAE*

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Досліджено вплив трьох типів наноструктурованих металовмісних сапонітових глин на життєздатність пивних дріжджів Saccharomyces cerevisiae. Експериментальні дані показують, що досліджувані матеріали, незважаючи на те, що вони мають частки нанометрового розміру, не проявляють інгібуючу дію на організми. У дослідженні продемонстровано значне збільшення кількості клітин для S. cerevisiaewas за дії наноматеріалів. Найбільша концентрація клітин була зареєстрована за дії нанокомпозиту Nb-Sap-EtO в концентрації 2 мг/мл, у цих умовах кількість клітин було збільшено до 5 разів у порівнянні з контролем. За даними МТТ тесту спостерігається стимулювання активності мітохондріальних редуктаз у пивних дріжджах за впливу наноматеріалів, які містять у своєму складі ніобій в концентраціях від 0,5 мг/мл.

Key words: *brewer's yeast, nanoparticles, saponite, toxicity, cells, mitochondrial reductase.*

Statement of the problem. Nanosized and nanostructured materials have a major potential for a practical application in a large number of research fields, in the industrial production as well as in everyday life. However, at the nanoscale level many substances acquire new properties and therefore may become biologically very active, interacting cooperatively or detrimentally with various biologic processes. It raises a series of issues about the potential toxic effects of nanomaterials on living organisms as a consequence of either intentional or unintentional contact with them. Thus, the assessment of the biological toxicity, if any, for any newly-obtained nanosized or nanostructured materials and nanocomposites is a key issue which has to be clarified before the full integration in everyday life.

Several reviews have already focused on the possible risks of the nanotech- and nanoscience-derived products [26], assessing potential hazards towards aquatic species, invertebrates, algae, plants and fungi [21, 14]. Moreover, there is an increasing number of experimental evidences indicating absorption of nanosized particles into cells and tissues [11], their translocation into non-target organs [18], and various adverse effects, such as inflammation, genotoxicity, teratogenesis and carcinogenesis [16, 17]. Among numerous nanostructured materials currently produced, metal and metal oxide nanoparticles are considered the most potentially dangerous because of their ability to breach epithelial barriers [13]. The negative effect of such nanosized materials may act at least in four different directions: i) by means of the nanoparticle or the nanosized material itself [7]; ii) by dissolution of the nanosized material with the consequent release of active ions from it [12]; iii) by a combined effect of the nanoparticle and of the released ions, both contributing to the toxicity [9], and iv) by a «Trojan Horse» effect, leading to an uncontrolled intake of possible hazardous impurities on/in the material [15]. Some uncertainty also exists on the role of nanostructured material size and shape in determining toxicity: in fact, while it is almost generally accepted that the biological activity increases as the particle size decreases, some studies reported contradictory results on this topic [28, 29]. Although for some nanoparticles the toxicity has been reported [22, 24], nevertheless too little is known about the mechanisms of such effects. The scientific literature on nanotoxicology consists of around 800 publications, generally about the cytotoxic effects of nanomaterials in cell culture system (*in vitro*). For instance, several recent papers point to the uptake and accumulation of nanosized materials in the prokaryotic and eukaryotic cells [20, 1, 3, 10, 27] and their toxicity [8, 2, 4]. Cells of plants, algae, and fungi constitute a primary site for interaction and a barrier for the entrance of nanoparticles. Mechanisms allowing nanoparticles or nanosized crystallites to pass through cell walls and membranes are as yet poorly understood.

Goal of study. Our main goal was testing different nanostructured clay-based materials to evaluate their safety in the perspective of any further application in the field of the decontamination and abatement of chemical warfare agent. In the present study, we report the experimental data about the potential toxic effect of novel types of nanocomposites onto brewer's yeast *S. cerevisiae* cells.

Materials and methods of research. Three types of nanocomposites based on the phyllosilicate clay saponite were examined at different concentrations: H-Sap is a protonic acid form of saponite, prepared according to the optimized procedure and reported previously [19]; Nb-Sap-EtO is a type of Nb-containing saponite clay (composition: $(Na)_{0,81}Mg_6(OH)_4(Al_{0,81}Nb_{0,07}Si_{7,11})O_{20}\cdot nH_2O$ and a H_2O:Si ratio = 20:1), prepared by adapting and tuning a method offered in the literature for parent saponite systems [5]; Nb-Sap-Cl is a niobium-containing saponite. Later it was prepared as follows: 6,68 g of amorphous SiO_2 (99,8 %, Aldrich 381268) were dispersed in 50 µl of water containing 0,63 g of NaOH. The obtained gel was stirred for 1 h at RT. 3,20 g of $Al[OCH(CH_3)_2]_3$ (98 %, Aldrich 22041-8) and 24,86 g of $Mg(AcO)_2\cdot 4H_2O$ (99 %, Aldrich 22864) were added and the gel was stirred for further 4 h. At the same time 0,37 g of $NbCl_5$ (99,5 %, Aldrich 215791) and 5,6 g of tetraethoxysilane (TEOS, 98 %, Aldrich 131903) were dissolved in 500 µl of water. The final solution was added to the gel and stirred for 1 h. Then the gel was poured in a Teflon cup of a sealed autoclave (Anton Parr 4748) and heated in an oven for 72 h at 240 °C. After the hydrothermal crystallization the solids were filtered, washed with deionized water to neutrality and dried in an oven for 24 h at 100 °C.

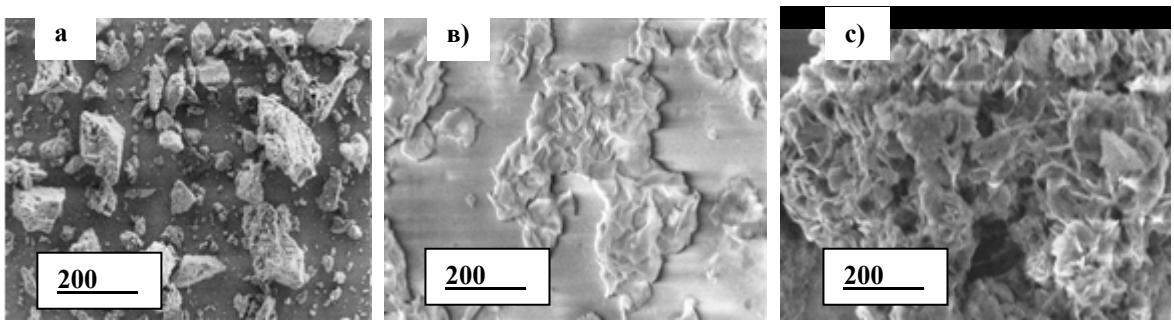
Scanning Electron Microscopy (SEM) was used to characterize the samples microstructure in a Leo 1550 Gemini SEM at operating voltage ranging from 10 kV to 20 kV and standard aperture value 30 µm. The study of the biocidal effect of the nanostructured materials was performed using brewer's yeast *S. cerevisiae*. The bioassay was performed using the following procedures.

Yeasts cultivation. Pure culture of *S. cerevisiae* strain U-517 from the collection of the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine was cultivated on the yeast extract peptone dextrose (YPD) medium during 1 night at 28 °C. The daily cultures at a concentration of 10^5 cells ml^{-1} in 25 ml of YPD medium at the certain concentrations of nanomaterials (0,5 mg ml^{-1}, 1,0 mg ml^{-1}, 1,5 mg ml^{-1}, 2,0 mg ml^{-1}) were incubated for 24 h. The number of cells have been counted in the Goryayev camera [6].

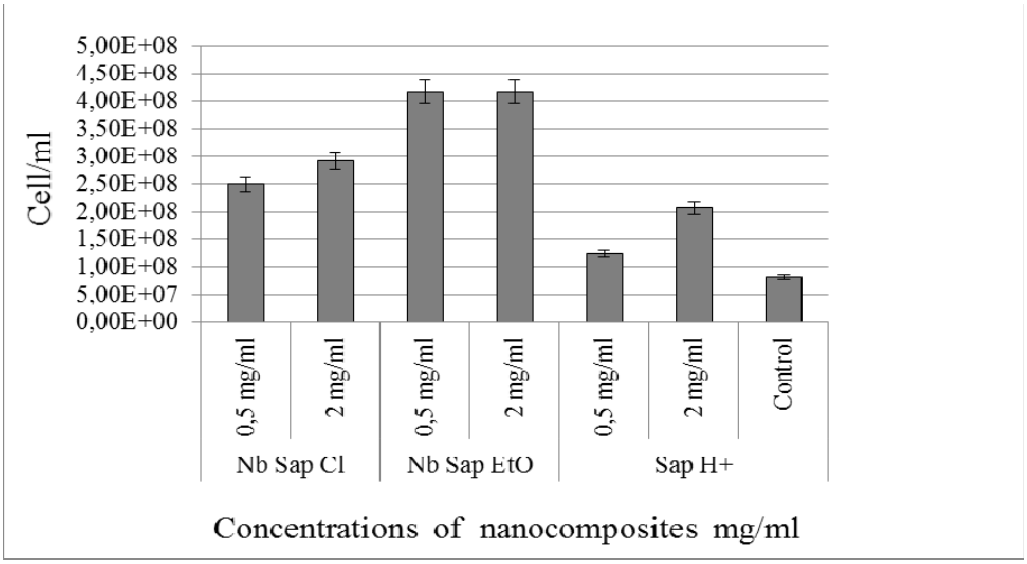
MTT assay. After daily processing of the cells by the materials to be tested, 100 µl of yeast culture were incubated in the presence of 20 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at the concentration of 5 mg ml^{-1} during 3,5 h at 37 °C in 96-well plates. It was repeated three times as minimum. After incubation the culture medium was removed and formazan (3-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan) crystals dissolved in 150 µl of DMSO. At last, the cells were incubated in the presence of DMSO in dark for 15 min, after they were measured to determine optical density formazan at a wavelength of 620 nm [25].

Results and discussion. The microstructures of the clay samples studied by SEM are presented in picture 1. The H-Sap samples have a shape that reflects the tetragonal structure and at the dissolution they agglomerate into large domains remaining porous with a pore size of 100 nm that indicated a substantial area of the active surface. The Nb-Sap-EtO sample was in the form of platelets 20 to 30 nm thick. Nb-Sap-Cl aggregates had a size of *ca.* 30 nm and, as the previous ones, were able to coalesce with the formation of larger domains.

It has been observed that under the influence of the solid samples the number of *S. cerevisiae* cell increased significantly (Pic. 2).



Pic. 1. SEM images of the nanocompositions: а) H-Sap, в) Nb-Sap-EtO, с) Nb-Sap-Cl

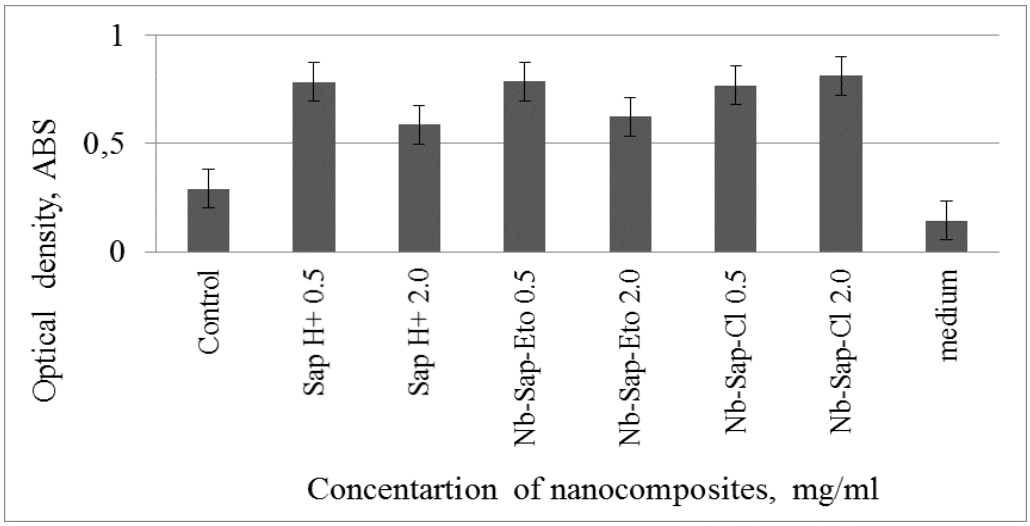


Pic. 2. Biological effect of saponite-based clays (H-Sap, Nb-Sap-EtO, and Nb-Sap-Cl) on the growth of S. cerevisiae cells number

The greatest effect is observed in the presence of Nb-Sap-EtO at a solid dispersion content of 2 mg ml^{-1}, where a cell concentration of 4,17 * 10^8 cells ml^{-1} was achieved, which corresponds to an almost 5-fold increase in their numbers compared to the control.

This fact can be explained by the remarkable redox and acid-driven hydrolytic properties of niobium-containing saponites [23]. Such features have been successfully exploited for catalytic purposes. In these tests the Nb-containing clays (in particular Nb-Sap-EtO) were likely able to accelerate the digestion and degradation of micronutrient complexes into simpler compounds. Therefore, the absorption and assimilation of nutrients by the yeast could take place at a lower energy cost and it resulted to be faster and more efficient.

As far as the results of the MTT test are concerned (Pic. 3), the most pronounced effect compared with the control has been recorded for H-Sap and Nb-Sap-EtO, at a concentration of 0,5 mg ml^{-1} as well as for the Nb-Sap-Cl sample at a concentration of 2,0 mg ml^{-1}. In the presence of these clays the cell number was higher up to *ca*. 4 times with respect to the reference level. When H-Sap and Nb-Sap-EtO were added to the yeast cells in a concentration of 0,5 mg ml^{-1}, the optical density were 0,7845 and 0,7852 respectively. Interestingly, by increasing the concentration of the clays in the culture medium, the effect was slightly smaller, but still greater than the control.



Pic. 3. Determination of the effect of the nanocomposites on the some biochemical properties of S. cerevisiae cells

Conversely, in the presence of Nb-Sap-Cl at a concentration of 2,0 mg ml^{-1}, it was as high as 0,8137. Such behaviour can be attributed to the remarkable hydrolytic and acid properties of the tested saponite clays (either with or without niobium), which can stimulate the mitochondrial reductases in the yeast cells.

Conclusion. The effect of acidic and niobium-containing saponite clays on the cells in the culture of *S. cerevisiae* was studied and registered. The most effect has been observed in the presence of Nb-Sap-EtO, a niobium-containing clay is prepared from niobium ethoxide, when at the concentration of 2 mg ml^{-1}, when the number of cells increased by 5 times compared to the reference level in the control.

According to the MTT test with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), the most relevant effect, with respect to the control tests, was recorded in the presence of acidic H-Sap and the hydrolytically-active Nb-Sap-EtO sample at the concentration as low as 0,5 mg ml^{-1}. They were able to stimulate the activity of mitochondrial reductases in yeast cells.

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