THE STUDY OF NEWLY SYNTHESIZED SAPONITE – BASED NANOCOMPOSITES INFLUENCEON THE BIOLUMINESCENT BACTERIA DEVELOPMENT

M. V. Taran, graduate student*

A.M. Kacev, doctor of biological sciences

K.E. Shavanova, candidate of biological sciences

M.F. Starodub, doctor of biological sciences

E - mail: TaranMaruna@gmail.com

Investigated of biological action of new nanomaterials basis on Saponite ($Si_{7.34}$ ($Al_{0,66}Mg_6$ O_{20} ($(OH)_4$) on development of bioluminescent bacteria. The studies of sharp and chronic biological action of water suspensions of standards conducted with the use of three basic kinds luminescent bacteria: *V.fischeri F1, P.leiognathi Sh1* and *V.harveyi Ms1*, line the action of nanomaterials was investigated on P.leiognathi of Sh1 by the method of diffusion in an agar. Coming from the conducted experiments, it is possible to draw conclusion about absence or presence of very weak toxic properties of the investigated nanopreparations.

Bioluminescent bacterium, nanomaterials, saponites, sharp toxicness, chronic toxicness

We live in the XXI century that began the rapid development of nanotechnology. [1, 2] Today, advanced economies are oriented to the development and application of nanotechnology as a promising industry in today's information world. Specific properties nanomaterials open opportunities for their practical application in many fields of science and industry, in particular when creating new efficient catalysts for the petrochemical industry for the manufacture of advanced sensor systems, structural ceramics and adsorbents, medicine and pharmacology for diagnosis and treatment of infectious and oncological diseases for materials with antibacterial properties, in agriculture of preparations for the protection and growth of plants and animals, environmental protection to detoxify chemicals, the use of nanoparticles as components of filters for water treatment [2, 3]. In crop nanopreparats use as micronutrients, provides increased resistance to adverse weather conditions and increasing yields of food and industrial crops. The effect, according to the authors [4], achieved through more active penetration of elements in the plant

through nanoscale particles and their neutral charge. [5]

All of the above scope of nanomaterials very promising, but we need to remember about the possible toxic properties of these structures. It is therefore necessary before providing recommendations for implementation nanomaterials the industry must make their toxicity tests on different biolodgical objects.

The aim of our study is to evaluate the toxicity of nanomaterials the example bacterial test systems.

MATERIALS AND METHODS

In the investigations were included the next samples of nanocompositions on the basis of Saponite ($Si_{7.34}$ (Al_0,$_{66}Mg_6O_{20}(OH)_4$) produced by IMV CAS #1319-41-1 (Nevada, USA): Saponite-H+; Nb-Sap-Cl and Nb-Sap-EtO .

The biological effects of the NPs and NC were studied in form colloidal water mixtures obtained in appropriate volumes of 3% of sodium chloride. Three strains of luminescent bacteria (*V. fischeri* F1, *P. leiognathi* Sh1 and *V. harveyi* Ms1) from collection of Crimean State Medical University (Simferopol', Ukraine) [7] as well as vegetables of *Phaseolus vulgaris L* were used as test objects. General scheme of biotesting acute and chronically toxicity in case of the application of luminescent bacteria is shown in Fig. 1. [6, 7]

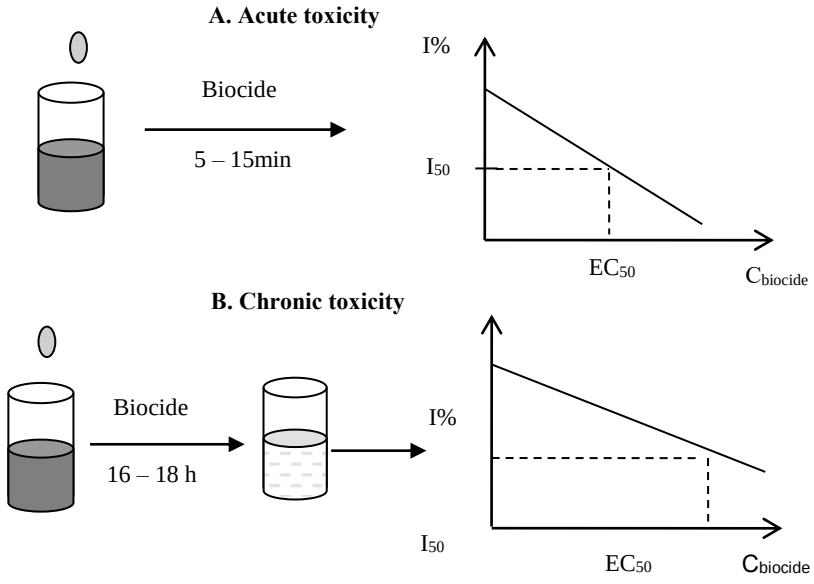

Fig. 1. Methodic for the determination of acute and chronic toxicity with the using bioluminescent bacteria.

The preparation of bacteria for the biotesting was made by the next way. The museum culture which was kept in the semi liquid agar under paraffin oil at first was

inoculated into the liquid medium. After incubation at the temperature of 25-30 ^{0}C during 24 h the samples were analyzed on the bioluminescence availability. In case of it presence bacteria were transferred from a liquid nutrient medium inoculated onto a solid one with sieving to separate colonies. For the next formation of subculture the most brightly glowing colony were reseeded in the liquid medium, which in the future (after a certain phase of growth, controlled by the growth time) were used for the bioassay.

For biotesting accomplishing the bacterial culture was diluted with 3% sodium chloride in 100 times (it was chosen experimentally depending on the brightness of bacterial bioluminescence). For the determination of acute toxicity of some samples it was mixed 50 μl bacterial culture with 0.8-0.9 ml testing solution dissolved in 2.5-3.0% of solution of sodium chloride and 100 μl of buffer solution (phosphate or tris-HCl) with pH of 7.0. The changes of bioluminescence were registered at the temperature of 28-30 ^{0}C by special device with recorder.

The biological effects of the analyzed samples were estimated according to character of the graphical dependences of the bioluminescence intensity on their concentration. At the presence of the expressed concentration inhibition or increasing bioluminescence, it's decreasing up to 50% or less was testified as toxic effect. Quantitatively, the level of toxicity was presented in the form of effective concentrations (for solutions of the individual substances) or dilution (for unknown composites) which could be able to arise decreasing bioluminescent intensity of suspension of bacteria up to 50% (EC_{50}). For construction of the appropriate curves it was analyzed the toxicity at the different concentration of the analyzed substances choosing their intervals in some preliminary experiments. In all cases the results of measuring bioluminescence were calculated with help the next formula: [6]

$$I(\%) = \frac{I_i}{I_0} \cdot 100$$

Where: I_0 and I_i– the level of the luminescence in the presence of sample and in the control, respectively.

The obtained data were presented graphically with the help of computer program Excel (Microsoft office XP). To determine appropriate effective concentrations it was used setting minimal values (50%) on the ordinate axel. At that,

the points of the intersections with the abscissa are corresponded to values of effective concentrations (EC_{50}). Chronic toxicity was determined according to effect of testing objects on the level of bioluminescence and growing bacteria. In this case it was made the similar as at the determination of acute toxicity, namely: bacteria were placed in luminometer cuvette for testing bioluminescence and after that 20-50 μl of nutrition medium was added to them and left for 5-16 h at the temperature of 25-30 $^{\circ}$C (dependence on optimum for used bacteria strains). Measurements of bioluminescence were made time to time and graphical dependences of its intensities on concentration of the testing substances were plotted as it was in case of the determination of the acute toxicity.

To analyze effect of nano-particles on the growing bacterial culture it was transferred on the solid nutritional medium. In this case 200 μl of the bacterial culture was equally dispersed on the agar surface and then it was covered by 10 mg of nano-particles. The presence of toxic or anti-bacterial effect was estimated according to the dimension of the zones of grows inhibition or changes in the intensity of luminescence though 18-24 h of cultivation.[6,7,8,9]

RESULTS AND DISCUSSION

It is necessary to underline that at the mixing NPs powder with the sodium chloride solution the formation of the colloidal system with uniform distribution was not revealed. The suspensions after shaking have formed precipitations.

The typical dependences of the intensity bacterial bioluminescence (I, %) on the concentration of NCs in the solution are given in Fig. 2. There is needed to mention the very small tendencies to decreasing of bioluminescence up to 80% in case of the increasing concentration to 2 mg/ml. This effect was not increased dependence on time of influence. But in case of the incubation of all strain bacteria with Nb-Sap-EtO NCs at the concentration of 1 mg/ml and during 1 h it was shown a small increasing level of bioluminescence (Fig. 2D). In general, in case of the investigations of acute toxicity of Nb-Sap-Cl and Nb-Sap-EtO NCs it was not observed any very strong oscillations in the intensity of bioluminescence at the 60 min of time incubation that maybe connected with some biochemical abilities of niobium as a metal ion (Fig. 3).

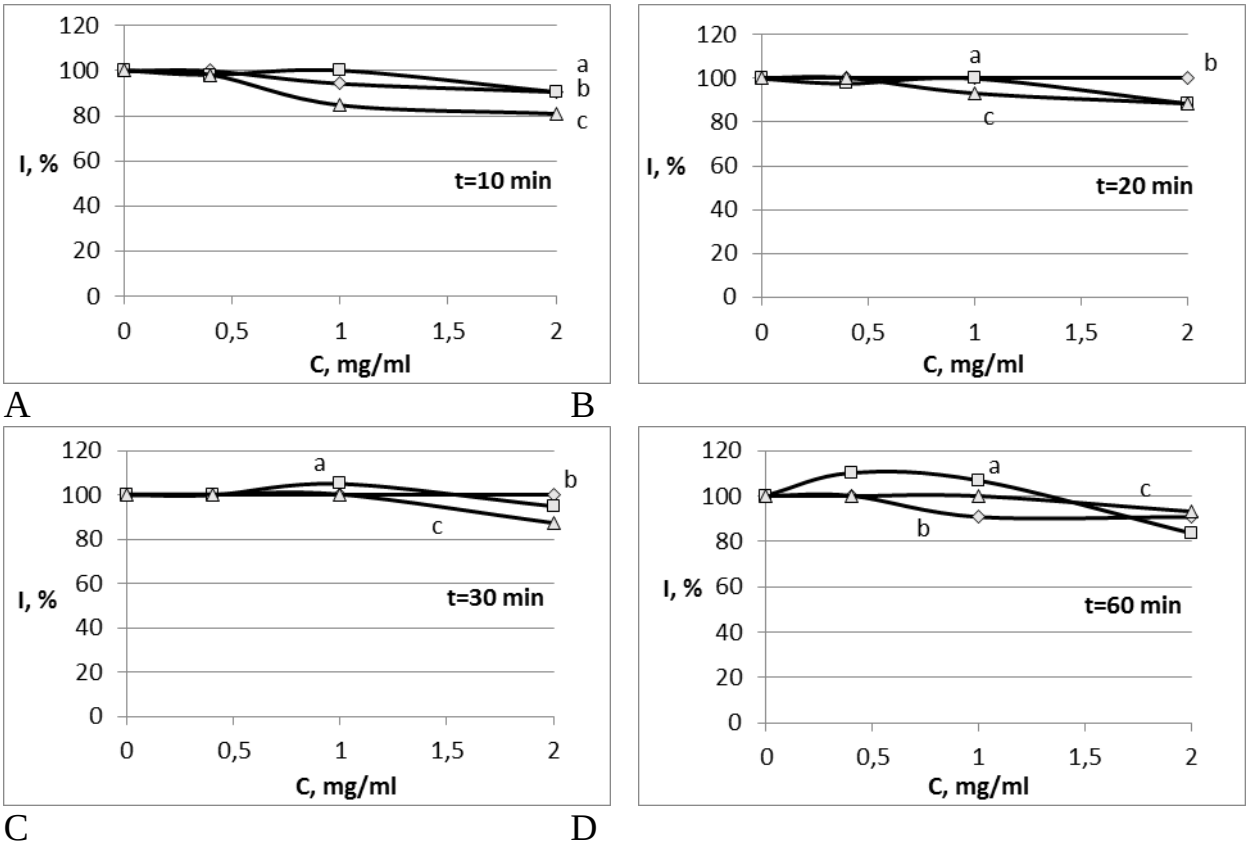

Fig. 2. Acute biological effect of the NCs on *P.leiognathi* Sh1 bacteria. A-D - time (10, 20, 30 and 60 min) of effect on the bioluminescent bacteria. Description: a - Nb-Sap-Cl; b - Saponite-H+; c - Nb-Sap-EtO.

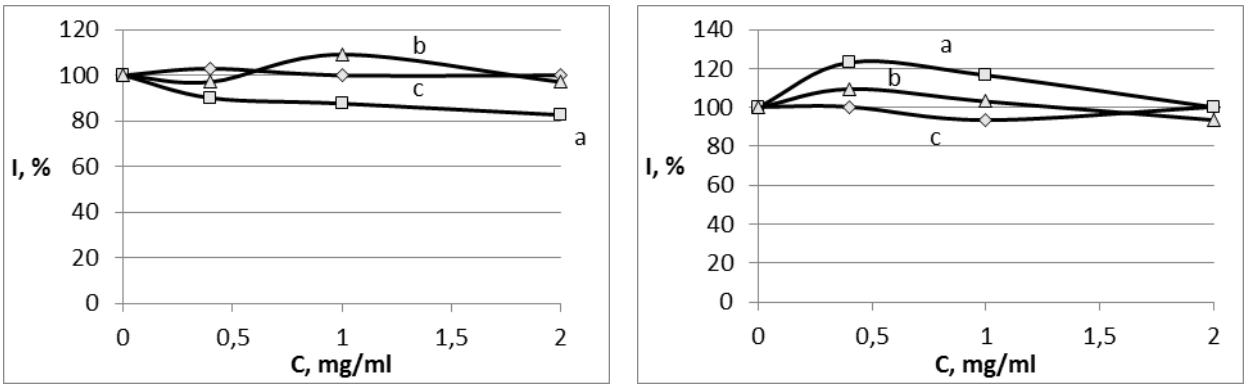

Fig. 3. Acute biological effect of the analysed NCs on the different strains of bioluminescent bacteria (time incubation was 60 min).

A - *V.fischeri* F1; B - *V.harveyi* Ms1. Description: a - Nb-Sap-Cl; b - Saponite-H+; c - Nb-Sap-EtO.

Taking into attention that nevertheless on the some mention effects the significant changing (decreasing or increasing) bacterial luminescence was not registered we can conclude about absence any acute toxic abilities of the analyzed samples. The investigation of chronic effect of NCs gives possibility revealing toxicity connected with the influence on the bioluminescence of bacteria and

biosynthetic processes accompanying division of cells and their growing. The greatest changes of bacterial bioluminescence at the influence of analyzed samples were observed at achieving their concentration up to 350% (Fig. 4). But any definitive patterns according chronic effects of the above mentioned NCs was not shown.

In some experiments it was registered the concentration depended inhibition of bioluminescence which was not be revealed at the repetition of analysis and, moreover, replaced by its activation. According to our opinion the obtained results in respect of chronic toxicity of the analyzed NCs may be connected with the specificity of growing bioluminescent bacteria in heterogenic systems connected solid particles. In such conditions bacteria may form complicate multi-cell structures, for example, biofilms in which the intensity of bioluminescence is regulated by specific genetic mechanisms. It is much known [17] that biofilms may be regulated by the “quorum sensing” mechanism which have higher stability to effect of number non-favorable factors of environment in the comparison with free bacteria in the suspension culture.

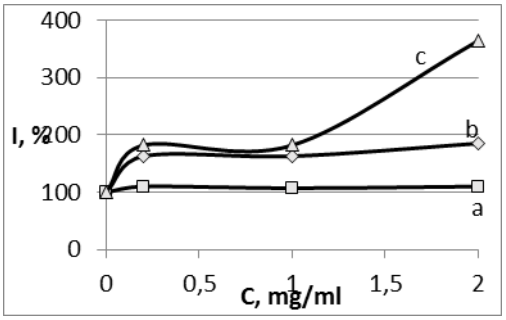

Fig. 4. Chronic effect of a different NCs on bacteria of *P. leiognathi* Sh1 (time incubation – 24 h). Description: a - Nb-Sap-Cl; b - Saponite-H+; c - Nb-Sap-EtO.

The taking into account of the obtained results and existed data of literature [18] about abilities on NPs to penetrate through membranes we investigated direct effect of the studied samples on the growing and bioluminescence of bacteria. The investigation fulfilled according procedure described above showed that around situation of the above mentioned materials (at the layer in 5 mg) any zones with the inhibition of bacteria growing was not observed during 18-24 h. Moreover, at this time it was registered the homogenous field luminescence on the all surface and without any changes near presence of the investigated samples .

CONCLUSIONS.

In studies of acute and hronic action nanomaterials Saponite-H + and Nb-Sap-Cl bacterial luminescence inhibition was not observed, and the use nanopreparat Nb-Sap-EtO at concentrations up to 1 mg / ml marked a slight increase bioluminescence. Research on the direct effect of nanomaterials bioluminescent bacteria confirmed the preliminary results, the toxic effects were found.

Based on the experiments, we can conclude the absence or presence of very weak nanopreparats studied toxic properties, allowing them to use in detoxification of chemicals.

REFERENCES

1. Balabanov, V. I. 2009, Nanotechnology, Science of future. M.: Eksmo, pp. 215–220 (in Russian).

2. Chekman, I. S. 2009, «Nanoparticles: Properties and prospects of use», Ukrainian bichemical journal, Vol. 81, no.1, pp. 122–129 (in Ukrainian)

3. Трахтенберг І. М. Наночастинки металів, методи отримання, сфери застосування, фізикохімічні та токсичні властивості / І. М. Трахтенберг, Н. М. Дмитруха // Український журнал з проблем медицини праці. - 2013. - № 4. - С. 62-74. - Режим доступу: http://nbuv.gov.ua/j-pdf/Ujpmp_2013_4_8.pdf

4. Бовсуновский А.М. Нанотехнологии как движущая сила аграрной революции / А. М. Бовсуновский, С. О. Вялый // Зерно. – 2008. - №11(31)С. 24-28.

5. Лопатько К.Г. Застосування наночастинок металів – екологічно безпечна технологія вирощування пшениці озимої / К.Г. Лопатько, Є.Г. Афтанділянц, О.В. Зазимко, С.М. Каленська, Л.М. Гончар, В.В. Трач, С.М. Захарченко // Науковий вісник НУБіП України, 158 - 2011р.

6. Кацев А.М. Биоаналитические аспекты использования светящихся бактерий в медико-биологических исследованиях 14.01.32 – медицинская биохимия, диссертация на соискание степени доктора биологических наук// А.М. Кацев, Симферополь-2013,389с.(С.93-97)

7. Биолюминесцентный микроанализ на основе бактериальной люциферазы и светящихся бактерий / [В.А. Кратасюк, С.Е. Медведева, Н.С.

Кудряшева, Е.Н. Есинбекова] // Очерки экологической биофизики: Юбл. сб. к 75-летию акад. И.И. Гительзона. – Новосибирск .: СО РАН, 2003. – с. 174-186.

8. Герхардт Ф. Методы общей бактериологии: в 3т. / Ф. Герхардт. – М: Мир, 1983. – Т.1. – 1983. – 536с.

9. Алешина Е.С. Использование люминесцирующих микроорганизмов для биотестирования минеральных вод: дис. канд. биол. наук : 03.00.07 / Е.С. Алешина. – Оренбург, 2008. – 182 с.