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Influence of metal containing nanocomposites on the kinetics of microbial population development

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Abstract. Magnetic nanoparticles are promising nanomaterials for biomedical applications. Studies have been conducted to study the effect of iron-containing nanoparticles at concentrations of 1.0 and 10.0 maximum tolerated dose for total iron on the growth and development of the microbial population of E.coli. On the basis of the obtained results, it was found that nanoparticles affect the growth characteristics of E.coli, related to human gram-negative prokaryotic microflora. The level of biological activity of nanoparticles depends on their concentration.

1. Introduction

Magnetic nanomaterials attract special interest due to their unusual properties and possible applications in biomedicine [1-3]. Nanocomposites in contrast with their bulk analogues represent a new factor of anthropogenic origin, to actively affecting both living organisms and their habitat [3-4]. In this regard, the question about the effect of magnetic nanoparticles (MNPs) on the nature of the interaction in the ecological system “human- microflora” is of considerable interest as the normal microflora performs strictly specific duties in maintaining homeostasis by performing strictly specific functions of each of them [5].

It is also worth to mention that environmental pollution with magnetic nanoparticles is steadily increasing. However, at present there is no standard methodology for iron-containing MNPs in order to determine their maximum tolerated dose (MTD). The level of iron regulated in water by sanitary-hygienic standards [6]: the MTD is 0.3 mg/l of total iron, but the definition of the maximum tolerated dose refers to soluble forms of iron. Nanoparticles, in a broader sense, are colloidal particles, and therefore they can not be considered as a soluble form. The particles are a separate insoluble phase with an interface with the aqueous medium. Although there is some solubility of iron at the boundary of the particle, most likely it does not exceed the MTD. The existing ambiguity in determination of the maximum allowable iron concentration in the case of MNPs stimulates the use of a wide range of methods for their careful physical and chemical certification. In this regard, we are working on a series of experiments aiming to determine the biological activity of iron oxide, in order to compare their effect on a similar model with nanoparticles.



The foregoing has determined the purpose of the present work - to study the adaptive capabilities of normal human microflora in the presence of iron oxide (γ -Fe₂O₃ maghemite) nanoparticles obtained by electrophysical technique of laser target evaporation.

2. Materials and methods

Magnetic MNPs of iron oxide (γ -Fe₂O₃ maghemite) were obtained by highly productive laser target evaporation technique (LTE) [7-9]. The X-ray diffraction structural characterization (XRD) was performed by DISCOVER D8 diffractometer with Cu-K α radiation. Accurate fit for the profile and identification of the Miller indices of the peaks for the description of the phase composition of nanoparticles was done using TOPAS program. The average size of coherent diffraction domains was estimated using the Scherrer approach [10]. Transmission electron microscopy studies (TEM) of MNPs were performed using a JEOL JEM2100 microscope.

The chemical composition of LTE MNPs was determined by the combination of Red-Ox titration and the analysis of the lattice period provided by XRD and TEM. As the biomedical applications demand magnetic MNPs in the form of water-based ferrofluids the suspension of air-dried MNPs in 5 mM sodium citrate was deaggregated by ultrasound treatment for 30 min using Cole-Parmer CPX-750 processor operated at 250 W. Permanent cooling of the suspension was provided. The remained aggregates were eliminated by centrifuging at 8000 rpm for 5 min. The de-aggregation of MNPs in suspension was monitored by the measurement of the hydrodynamic diameter. The hydrodynamic diameter of MNPs by DLS using Brookhaven Zeta Plus analyzer. The iron ions concentration was defined after suspension preparation. As soon as the composition of MNPs was carefully defined by titration (Fe_{2.7}O_{4.0}) we simply recalculated the amount of iron ions taking into account the initial concentration of suspension (2.44 wt. % of MNPs).

Magnetic measurements of the air-dry MNPs and MNPs dried from the suspension were carried out with MPMS XL-7 SQUID-magnetometer. Magnetic measurements confirmed that MNPs were close to a superparamagnetic state with low coercivity of about 20 Oe and saturation magnetization defined as a magnetization at 20 kOe field of 35 emu/g at room temperature.

The biological studies were performed with an experimental model under conditions of periodic cultivation, where the reference E.coli strain belonging to the normal human microflora was used as a test object. In the experimental and control cases, the same test object culture synchronized for the experiment was used. In the control case, the microbial population was grown in standard meat-peptone broth (BMP), pH 7.4 \pm 0.4. In the experimental case, a suspension of iron oxide MNPs was introduced into the BMP, reaching their concentrations of 1.0 and 10.0 maximum tolerated dose (MTD) for total iron. Maximum tolerated dose was defined as 0.3 mg/L iron ions in water solution. The microbial suspension exposition was 144 hours both in the experiment and in the control cases.

In order to determine the dynamics of the development of the microbial population under control and experimental conditions the time of the starting point was set to zero. Afterwards quantitative sectoral seeding by the method of Gold from the culture fluid was carried out every 24 hours with seeding onto meat-peptone agar (MPA) solid nutrient medium in order to define the concentration of microorganisms in a periodic culture [11]. The cultivation conditions were standard and corresponded to the growth and development of the microbial population of E. coli. Based on the obtained results, the periods of its development were graphically determined and growth indices were calculated under control and experimental conditions.

3. Results and discussion

The values of weighted averaged diameters obtained by TEM and by XRD were compared to each other. They were quite close to each other. As to expect, the experimental XRD data were well fitted by the magnetite data base. Exact chemical composition of MNPs was determined by the traditional method of combination of Redox titration and the lattice period analysis available with XRD data. The lattice period (a) of MNPs was somehow lower than one typical for stoichiometric magnetite but higher than the lattice parameter for γ -Fe₂O₃: a = 8.358(5) Å. Average size calculated using data for

TEM images was as high as 14(3) nm, and calculated with Sherrer formula from XRD data was as high as 13(2) nm.

Figure 1 shows the thermomagnetic curves (ZFC-FC) obtained by cooling without applying a field (ZFC) and cooling in a magnetic field $H = 100$ Oe (FC) for the temperature (T) range of 5 to 300 K. Magnetic measurements give information on the magnetic state of nanoparticles. Although in the present work we do not have a specific goal to evaluate applicability of this particular kind of nanomaterials for magnetic biosensing or targeted drug delivery [12], the obtained results can be useful for the development of the present research line in a future.

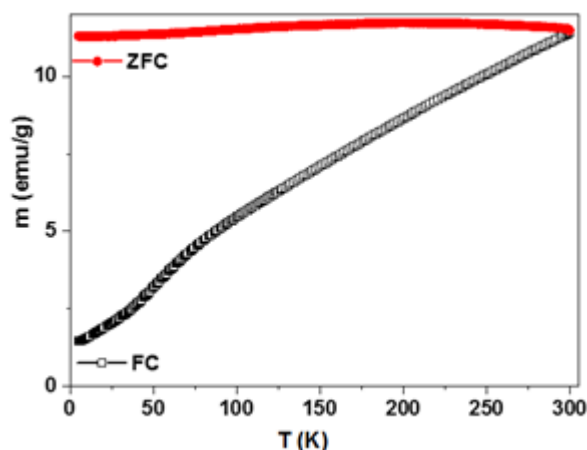


Figure 1. Thermomagnetic curves (ZFC-FC) obtained by cooling without applying a field (ZFC) and cooling in a magnetic field $H = 100$ Oe (FC) for γ - Fe_2O_3 maghemite nanoparticles obtained from dried suspension

One can see that no maximum on the ZFC-FC curve is present and ZFC and FC curves are significantly differ from each other. Observed features indicate that the γ - Fe_2O_3 maghemite MNPs are in the blocked state [13]. Magnetic methods are very useful for detailed characterization of MNPs.

In accordance with the goal of this research, among the factors influencing the nature of the development of the microbial population, it is necessary to analyze both food resources that determine the trophic properties and the effect of iron oxide nanoparticles. Other factors that can affect the development of the microbial population can be neglected, since in the control and experimental variants remain stable. Experimental studies with one batch of nanoparticles were delivered in triplicate of one experiment.

In the analyzed period the culture liquid was sampled from the experimental and control variants and quantitatively sown according to the Gold method on MPA solid nutrient medium in order to determine the concentration of microorganisms in periodic culture. The main feature of periodic cultivation is that during the experiment the nutrient substrate is not additionally introduced. The microbial population is self-developing in a limited volume of the medium without additional nutrients. To simulate the influence of the agent under study in different physiological periods of growth and development of the culture of microorganisms. Quantitative inoculation of the nutrient medium MPA is necessary in order to determine the period of physiological growth of the culture. Comparison of the CFU/ml indices in the control and experimental groups allows evaluate the influence of the studied factor on the development of the microbial population. CFU - colony-forming units, that is, the number of grown colonies in the experiment and control expressed by the unit of measurement \lg CFU / ml of culture fluid. Quantitative sowing of culture liquid on the nutrient medium allows not only to fix the presence of viable microorganisms in it, but also to determine the density of the population of microorganisms developing respectively in the control and conditions of the experiment.

Based on the obtained experimental data results (figure 2), it was found that the nature of the growth curve of the microbial population of *E. coli* in the control case has a typical dependence for the periodic culture, but the stage of death for 144 hours of the experiment does not occur. This indicates that the trophic needs of *E. coli* are fully met by the potential nutrients that are present in the substrate.

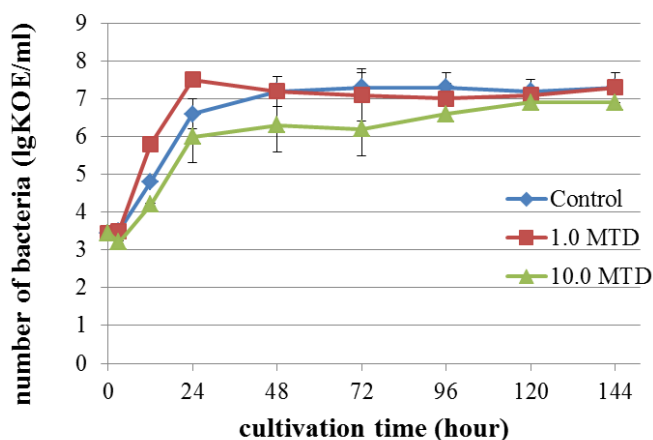


Figure 2. The dynamics of growth and development of the microbial population of E.coli in the presence of γ -Fe₂O₃ maghemite nanoparticles in different equivalents of maximum tolerated dose.

The analyzed microbial population after 3 hours of cultivation enters the exponential grows stage, which lasts 45 hours (table 1). During this period, the concentration of microbial cells reaches its maximum lgCFU (Colony Forming Units - indicator characterizing microbiological the degree of bacterial contamination) of 7.2 cells/ml with a specific growth rate and a constant for cell division, respectively, 0.35 and 0.49 h⁻¹ (table 2).

Table 1. The development of a periodic E.coli culture under the influence of γ -Fe₂O₃ maghemite nanoparticles.

MTD calculated on the basis of total iron content in MNPs	Time of development of periodic culture by growth stages (hour)		
	adaptation	exponential grows	stationary
control	3.0	45.0	96.0
1.0	3.0	21.0	120.0
10.0	3.0	21.0	120.0

Table 2. Indicators of growth and development of the microbial population of E.coli in the period of exponential growth, experiencing the influence of γ -Fe₂O₃ maghemite nanoparticles.

Analyzed case	Growth rates for periodic culture:			
	Specific growth rate (hour ⁻¹)	Number of cell divisions	Generation time (hour)	Coefficient of division rate (hour ⁻¹)
control	0.35	10.6	2.04	0.49
1.0 MTD for total iron	0.45	13.6	1.59	0.63
10.0 MTD for total iron	0.31	9.3	2.33	0.43

The indicator characterizing the doubling time of the microbial cell was 2.04 hours. At the end of the exponential stage, when the microbial population thickens in the culture fluid, its growth rates decrease 6.0 times, while the generation time increases. This indicates that the population has entered a stationary period in the conditions that hinder its development, as a result of which the cell

concentration remains stable at the same level. A feature of the exponential stage of development of the test culture in the control is the presence of a period of growth retardation, which lasted 24 hours, which is typical of normal physiological conditions. Change in the size of viable cells in the microbial population was not observed for the next 96 hours from the end of the exponential stage of development of the periodic culture. This indicates that it has entered a period of stationary growth, when the microbial population thickens in the culture fluid, because of which its trophic functions were impaired. In addition, toxic microbial metabolism products and destroyed cells accumulated in the culture medium. All circumstances created conditions that inhibited the development of the microbial population, i.e. typical for the stationary period of development behaviour was observed. During this period, the number of dead microbial cells corresponded to the number of newly formed, and therefore the cell concentration remained stable at the same level.

Experimental studies on the growth and development of the microbial population of *E. coli* in the control case provided an opportunity to assess the quality of life and compare its growth rates against the background of the action of lgCFU index. It was found that the growth curve in the experimental cases has the same type as that one for the control case (figure 2). The adaptation phase in experimental cases, which covers the period between the inoculation of microorganisms into the nutrient medium and the exponential growth phase, lasted as long as for a control case for 3 hours (table 1).

Unlike the control sample, in the experimental samples the exponential phase of the development of the test culture ends 24 hours earlier and has no period of slowing growth rate before the period of stationary development of the periodic culture. The maximum value of lgCFU in the culture fluid is 7.5 and 6.0 cells/ml, which corresponds to 1.0 and 10.0 MTD of γ -Fe₂O₃ maghemite nanoparticles. In the experimental case with the minimum concentration of the agent under study, it was noted that after the exponential growth stage was completed, the lg CFU index was decreased. However, after 24 hours of cultivation, lgCFU index was stabilizing at 7.0 cells/ml and the periodic culture of *E. coli* entered the stationary growth stage. In contrast to this behavior, for experimental case, when exposed γ -Fe₂O₃ maghemite nanoparticles at a concentration of 10.0 MPD for total iron on the microbial population of *E. coli*, this effect was not detected.

For the exponential stage of development of the microbial population, the parameters of growth and development of a periodic *E. coli* culture were calculated (table 2). It has been established that their values depend on the concentration of the active agent on the microbial population. So, if at high concentration they were only slightly different from the control value, then at the concentration of the test agent of 1.0 MPD for total iron they were 1.3 times higher, while the generation time decreases. This indicates that in low concentrations, iron-containing nanoparticles stimulate the process of reproduction, because of which the time of the cell cycle become reduced.

It observed that the exponential period of growth and development of a periodic culture determines the course of the stationary phase. In the cases of experimental and control samples, the microbial population did not go from the stationary growth stage to the death stage. At the same time, for the experimental case with a concentration γ -Fe₂O₃ maghemite nanoparticles of 10.0 MPD for total iron, a toxic effect of the action was observed, which was manifested in the death of the test culture. The lethal effect throughout the experiment was as high as 13.0%.

Based on the above presented analysis, it follows that in the presence of γ -Fe₂O₃ maghemite nanoparticles, the process of microbial adaptation takes place in the direction of enhancing their viability. Nanoparticles have a slight lethal effect on the population of *E. coli*. It can be assumed that the process of adaptation to the effects of γ -Fe₂O₃ maghemite nanoparticles will also develop along the path of variability of physiological and biochemical processes with the formation of defense mechanisms. As a result, due to the death of some, more adapted microbial cells survive, which makes it possible to evolve the species.

4. Conclusion

Magnetic nanoparticles of the iron oxide were obtained by the LTE technique. Their structure and magnetic properties were carefully analyzed. The results of experimental studies have shown that γ -Fe₂O₃ MNPs affect the growth and development of the microbial population related to human gram-negative prokaryotic microflora. The level of their biological activity depends on the concentration of MNPs. The weak stimulating effect was observed for the MNPs concentration of 1.0 MTD. The concentration of nanoparticles, corresponding to 10.0 MTD for total iron, showed an inhibitory effect on the test culture. The change in generation time during the period of exponential growth indicates that iron-containing nanoparticles affect the reproduction process.

Acknowledgments

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