13. Gorin D.A., Shchukin D.G., Koksharov Yu.A., Portnov S.A., Köhler K., Taranov I.V., Kislov V.V., Khomutov G.B., Möhwald H., Sukhorukov G.B. Effect of microwave irradiation on composite iron oxide nanoparticle/polymer microcapsules. *Proceedings of SPIE*, 2007, vol.6536, no. 653604.

14. Schwendener R.A. Liposomes in biology and medicine. *Adv. Exp. Med. Biol*., 2007, vol. 620, pp. 117-28.

15. Lasic D.D. Liposomes: from physics to applications. *Elsevier, Amsterdam*, New York, 1993, 575 p.

16. Wagner A., Vorauer-Uhl K Liposome Technology for Industrial Purposes. *Journal of Drug Delivery*, 2011, article ID 591325, 9 p.

17. Koning G.A, Eggermont A.M., Lindner L.H., ten Hagen T.L.M. Hyperthermia and Thermosensitive Liposomes for Improved Delivery of Chemotherapeutic Drugs to Solid Tumors. *Springer Pharm Res*., 2010, pp. 1750-1754.

18. Ranganathan1 R., Madanmohan S., Kesavan A., Baskar G., Ramia Y., Krishnamoorthy, Santosham R., Ponraju D., Rayala S.K., Venkatraman G. Nanomedicine towards development of patient-friendly drug-delivery systems for oncological applications. *International Journal of Nanomecine*, 2012, pp. 1043-1060.

19. Glaser R.W., Leikin S.L., Chernomordik L.V., Pastushenko V.F., Sokirko A.I. Reversible electrical breakdown of lipid bilayers – formation and evolution of pores. *Biochimica Et Biophysica Acta*, 1988, vol. 940, pp. 275-287.

20. Weaver J.C., Chizmadzhev Y. Theory of electroporation. *A review. Bioelectroch Bioener*, 1996, vol. 41, pp. 135-160.

21. Widder K.J., Senyei A.E., Scarpelli D.G. Magnetic microspheres:a model system for site specific drug delivery in vivo. *Proc. Soc. Exp. Biol. Med.*, 1978, vol. 58, pp. 141-146.

22. Giersig M., Khomutov G.B. (editors) Nanomaterials for application in medicine and biology. *Springer, Dordrecht*, The Netherlands, 2008, 188 p.

23. Amstad E., Textor M., Reimhult E. Stabilization and functionalization of iron oxide nanoparticles for biomedical applications. *Nanoscale*, 2011, vol. 3, no. 7, pp. 2819-2843.

24. Gupta A.K., Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials*, 2005, vol. 26, pp. 3995-4021.

25. Massart R. Preparation of aqueous magnetic liquids in alkaline and acidic media. *IEEE Transactions on Magnetics*, 1981, vol. 17, pp. 1247-1248.

26. Gulyaev Yu.V., Cherepenin V.A., Vdovin V.A. [et al.] Pulsed electric field-induced remote decapsulation of Nanocomposite liposomes with implanted conducting nanoparticles. *Journal of communications technology and electronics*, 2015, vol. 60, no. 10, pp. 1097-1108.

FLUORESCENCE TECHNIQUES FOR BIOINDICATION OF NANOMATERIALS TOXIC EFFECTS ON PHYTOPLANKTON

Matorin D.N., Protopopov F.F., Goryachev S.N., Bratkovskaja L.B.
Moscow State University
Vorobyevi Gory str., 1, 119892, Moscow, Russia
e-mail: matorin@biophys.msu.ru

Abstract. Perspectives of using fluorescence of chlorophyll methods for the assessment of toxic effects of nanomaterials on phytoplankton is analyzed. Measuring of light dependence curves of fluorescence allows changes in energy storage in photosynthetic processes in algal cells to be detected at early stages of phytoplankton exposure to modern nanomaterials.

Key words: nanomaterials, phytoplankton, chlorophyll fluorescence, photosynthesis, ecology.

Phytoplankton forms the basis of water ecosystems and determines their state and productivity. Various environmental factors and anthropogenic pollution first affect the concentration and photosynthetic activity of algal cells, in turn, changed photosynthetic activity of phytoplankton results in changes in other levels of aquatic ecosystems [1]. Changes in the amount and activity of algae in a waterbody, induced by anthropogenic factors, can markedly deteriorate the quality of aquatic environment. In some cases, this can present a direct threat for population health, owing to release of highly toxic substances by planktonic algae.

Characteristics of photosynthesis, which is the major energy storing process in algal cells [1, 2], can be used as an indicator of the physiological state of phytoplankton. Presently, the most pressing task is to develop methods, which allow early changes in the abundance and state of microalgae, induced by toxic pollutants to be continuously monitored in teal time before the visible signs of ecosystem damage appear. Such integral information on the state of environment can be obtained with bioindication methods, which are based on registration of the response of natural phytoplankton populations. Bioindication methods can be used for continuous monitoring of water quality and for rapid detection of an increase toxic pollutants (nanomaterials). After obtaining positive signal from bioindication of the environment for integral toxicity, analytical methods can be additionally used to identify the chemical nature of the pollution. Bioindication methods shold be informative, sensitive and operate in real time regime. Since algae form the base of aquatic ecosystems, they can be used as bioindicators of their habitats. The basic idea of the methods developed is research is that the fluorescence of chlorophyll, which is present in photosynthetic membranes, is a valuable source of information on the amount and state of algal cells [1, 3-5]. Fluorescence yield at a low intensity of exciting light (Fo)

was shown to correlate with the concentration of chlorophyll and biomass of microalgae. This characteristic is mainly determined by the total concentration of light-harvesting pigments in algal cells and is a characteristic of phytoplankton abundance [6, 7].

Measurement of variable fluorescence of chlorophyll (Fv) can be used to estimate the photosynthetic activity of algae. Photosynthetic activity of phytoplankton depends on the primary processes of light energy transduction into the energy of chemical bonds, which take place in photosystem II reaction centers (PSII RC), which are involved in water splitting and O_2 evolution. The value of Fv is generally determined as the difference between the maximum fluorescence yield, Fm, measured with closed (photochemically inactive) reaction centers, in which all light energy absorbed by pigments of photosynthetic apparatus is emitted in the form of fluorescence and is dissipated into heat, and the fluorescence yield Fo with open PSII RC, in which most part of the absorbed energy is used in photosynthetic reactions, and energy loss is at its lowest [1, 7]. Thus, variable fluorescence (Fv=Fm-Fo) is a measure of the energy which has been photochemically converted by PSII. The Fv/Fm ratio was shown to reflect the efficiency of photochemical conversion of light energy in PSII (further referred to as photochemical activity) algae and to correlate with photosynthetic rate measured from the rates of O_2 evolution or CO_2 fixation [8].

At the Department of Biophysics of the Faculty of Biology of the Moscow State University we designed a complex of luminescence methods for diagnostics of the physiological state of phytoplankton cells, which comprises a single-beam on-board fluorometer, a submersible pump-and-probe fluorometer, flow fluorometer, microfluorometer, and a device for measuring delayed fluorescence [3, 7, 9, 10].

Fluorescence of algal cultures can be successfully used as biosensor for testing nanomaterials [7, 11]. However, such work was not performed with natural phytoplankton.

In the present paper, we used fluorescence methods to study ecotoxicological effects of different nanoparticles on natural phytoplankton.

Materials and methods. Experiments were performed with natural phytoplankton from shore waters of the Black Sea. Chlorophyll concentration in samples was 1-0.5 μg l^{-1}. Samples were collected in the morning and were incubated with added nanoparticles under conditions similar to natural habitat (20 ^{0}C and illumination 30 μE/m^2 s).

We studied synthetic nanocomposite $Fe_3O_4/HA_{mech,}$ made from magnetite nanoparticles and humic substances, diamond nanoparticles (10 nm) [12], nanosilver preparation [*Ag*] (average radius 40 nm) and nanogold preparation [*Au*] (average radius 25 nm).

Fluorescence was measured with a portable pulse fluorometer MEGA-25 constructed at the Department of Biophysics, MSU [7; 10], and a WaterPAM fluorometer (Walz, Germany). The maximum quantum yield of photosystem II (PSII) Fv/Fm, where Fv=Fm-Fo, was measured on dark-adapted algae. Measurements under light illumination were performed with progressive increase of light intensity from 0 to 800 μE/m^2 s. At the end of each illumination session, Fm' and fluorescence yield in the light F(t) were measured using saturating flashes (0.8 s, 3000 μE/m^2 s) [8]. These parameters were used to calculate nonphotochemical quenching of chlorophyll fluorescence NPQ = (Fm-Fm')/Fm', quantum yield of photochemical conversion of light energy in PSII, as Y = (Fm'-Ft)/Fm' and relative yield of the noncyclic electron transport at given light intensity rETR = Y x E_i, x 0.5, where E_i – illumination intensity. The measured parameters are designated according to the generally accepted nomenclature [8].

Results. Fluorescence parameters showed the effect of various nanoparticles on natural phytoplankton (Table 1). The maximum quantum yield of PSII - Fv/Fm differentially decreased during the exposure to different nanomaterials. The number of cells and the parameter Fo, reflecting the abundance of phytoplankton [4], little changed during incubation time - 20 hours. Particularly significant inhibition of photosynthesis in cells was seen under the effect of colloidal solution, containing silver nanoparticle in the preparation «Argonica», recommended for medical use. The effect was noted at concentrations down to 10^{-8} M. Silver and nanodiamond nanoparticles affected phytoplankton. By contrast to silver, gold nanoparticles slightly affected photosynthetic activity of phytoplankton even at high concentrations. Synthetic nanocomposite, Fe_3O_4/HA_{mech} also slightly affected phytoplankton activity (Table 1).

Table 1 – Changes in fluorescence parameters - Fv/Fm – samples in darkness, $rETR_{max}$ – maximum relative rate of electron transport and NPQ = (Fm/F'm) - 1-nonphotochemical fluorescence quenching at illumination 800 μE/(m^2 s) in natural phytoplankton after the addition of various nanopreparations (incubation time – 20 hours) as percentage from the control value

Fluorescence parameters	Control	Nanogold [*Au*] $5 \cdot 10^{-5}$ M	Diamond nanoparticles (0.005%)	Nanosilver 10^{-5} M	Colloidal silver in preparation «Argonika» 10^{-7} M.	Nanocomposite Fe_3O_4/HA_{mech} (0.1%)
Fv/Fm	100%	93%	40%	70%	42%	80%
$ETR_{(max.),}$	100%	87%	20%	41%	27%	57%
NPQ	100%	110%	29%	61%	29%	76%

In another approach we used measurements of light dependence curves of various fluorescence parameters under a gradual increase of light intensity. This method has been actively developed in recent time for the investigation of photosynthesis in leaves and algae [13]. These dependences can be used to assess the relative rate of noncyclic electron transport rETR and to measure the nonphotochemical fluorescence quenching (NPQ) [4].

It was found that silver, gold and diamond nanoparticles, as well as nanocomposite Fe_3O_4/HA_{mech}, reduce the rate of noncyclic photosynthetic electron transport in phytoplankton, as calculated from data on fluorescence parameters. The reduction of ETR_{max} – (maximum relative rate of electron transport, as percentage of the control value) under the effect of nanoparticles is shown in Table. It is seen that this parameter, when measured in light, is more sensitive to the effect of nanoparticles, compared to Fv/Fm ratio. Nonphotochemical quenching of fluorescence at 800 $\mu E/m^2$ s is also reduced to a greater extent under the effect of nanoparticles, compared to Fv/Fm. Parameter NPQ increased only in the presence of gold nanoparticles.

Conclusion. Thus, our data shows that measurement of chlorophyll fluorescence is a promising method for the early detection of nanomaterials in natural aquatic environment. These methods are able to give information about the state of photosynthesis, the main process in algal cells, at early stages of the phytoplankton exposure to nanomaterials, before a damage caused to aquatic ecosystems becomes visible. Such approach can afford to implement environmental protection measures in proper time. Fluorescence measurements under varying light intensities load may increase the sensitivity of this method in detecting damaging effects of nanomaterials.

References:

1. Falkowski P.G., Raven J.A. Aquatic photosynthesis. *Malden: Blackwell Science*, 1997, 375 p.

2. Matorin D.N., Osipov V.A., Yakovleva O.V., Pogosyan S.I. *Assessment of the state of plants and algae based on chlorophyll fluorescence*. Guidance manual. Moscow: MSU, Max Press, 2010, 117 p.

3. Antal T.K., Venediktov P.S., Matorin D.N., Ostrowska M., Wozniak B., Rubin A.B. Measurement of phytoplankton photosynthesis rate using a pump-and-probe fluorometer. *Oceanologia*, 2001, vol. 43, no. 3, pp. 291-313.

4. Antal T. K., Matorin D.N., Ilyash L.V., Volgusheva A.A., Osipov V.A., Konyuhov I.V., Krendeleva T.E., Rubin A.B. Probing of photosynthetic reactions in four phytoplanktonic algae with a PEA fluorometer. *Photosynthesis Res.*, 2009, vol. 102, pp. 67-76.

5. Antal T.K., Osipov V.A., Matorin D.N., Rubin A.B. Membrane potential is involved in regulation of photosynthetic electron transport in the marine diatom *Thalassiosira weissflogii*. *Journal of Photochemistry and Photobiology: Biology*, 2011, vol. 102, pp. 169-173.

6. Matorin D.N., Antal T.K., Ostrowska M., Rubin A.B., Ficek D. Chlorophyll fluorometry as a method for studying light absorption by photosynthetic pigments in marine algae. *Oceanologia*, 2004, vol. 46, no. 4, pp. 519-531.

7. Matorin D.N., Karateeva A.V., Osipov V.A., Lukashev E.P., Seifullina N.Kh., Rubin A.B. Influence of Carbon Nanotubes on Chlorophyll Fluorescence Parameters of Green Algae *Chlamydomonas reinhardtii*. *Nanotechnologies in Russia*, 2010, vol. 5, no. 5-6, pp. 321-328.

8. Schreiber U. Pulse-Amplitude (PAM) fluorometry and saturation pulse method. In: *Chlorophyll fluorescence: A signature of Photosynthesis*. Papageorgiou G. and Govindjee (eds.), Springer, Dordrecht, Netherlands, 2004, pp. 279-319.

9. Matorin D.N., Venediktov P.S., Konev Yu.V., Rubin A.B. Application of a doublee-flash, impulse, submersible fluorimeter in the determination of photosynthetic activity of natural phytoplankton. *Doklady Russ. Acad. Sci*., *Earth Sci. Sect.*, 1996, vol. 350, no. 7, pp. 1159-1161.

10. Matorin D.N., Rubin A.B. *Chlorophyll Fluorescence in Algae and Higher Plants*. Moscow-Izhevsk: IKI-RKhD, 2012.

11. Wang J., Qiang Hu. Old bottle with new wine: algal indicators for nanotechnology-based toxicology. *Electronic Journal of Biology*, 2005, vol. 1, no. 1, pp. 9-13.

12. Terekhova V.A., Vavilova V.M., Fedoseeva E.W., Karateeva A.V., Matorin D.N., Gubarevich T.M. Detoxicating effect of humic preparations when used in combination with carbon nanoparticlesю *Proceedings of V All-Russian Conference «Humic substances in biosphere». Part 1 – SPb.*: Publishing house SPBGU, 2010, pp. 462-469.

13. Herlory O., Richard P. Blanchard G. F. Methodology of light response curves: application of chlorophyll fluorescence to microphytobenthic biofilms. *Mar Biol.*, 2007, vol. 153, pp. 91-101.