

DIAGNOSTICS OF ANOXYGENIC PHOTOTROPHIC MICROORGANISMS USING SPECTRAL METHODS: DEPTH DISTRIBUTION OF DIFFERENTLY PIGMENTED BACTERIA IN NATURAL LAKES AND ESTIMATION OF THE AMOUNT OF BACTERIOCHLOROPHYLLS PER CELL

Anna Zhiltsova¹, Anastasia Kharcheva¹, Pavel Norin², Elena Krasnova³,
Olga Lunina⁴, Alexander Savvichev⁴ and Svetlana Patsaeva¹

1. Lomonosov Moscow State University, Faculty of Physics, Moscow, Russia;
[a.a.zhiltsova\(at\)gmail.com](mailto:a.a.zhiltsova(at)gmail.com)
2. Lomonosov Moscow State University, Faculty of Biology, Moscow, Russia
3. Lomonosov Moscow State University, Faculty of Biology, Nikolai Pertsov White Sea Biological Station, Republic Karelia, Russia
4. Winogradsky Institute of Microbiology, Russian Academy of Science, Moscow, Russia

Nowadays the Arctic zone has a tendency to develop, which means expansion of a road network and construction of tidal power plants. Due to these factors some water areas will be artificially separated from the sea, undergoing changes in their ecological systems (among them are a stagnant phenomenon and a hydrosulphuric accumulation). In such a situation an environmental remote monitoring is necessary for which spectral methods can be employed, and where the anoxygenic phototrophic microorganisms including green sulphur bacteria may be of a particular interest (1). These bacteria are widespread in such areas because they are able to use hydrogen sulfide for photosynthesis.

Green sulphur bacteria are divided into two types according to their pigmentation: green-coloured bacteria containing bacteriochlorophylls c and d and carotenoid chlorobactene, and brown-coloured bacteria containing bacteriochlorophyll e and carotenoid isorenieratene. Due to different pigments the two groups of bacteria have different absorption spectra as well as fluorescence emission and excitation spectra (2).

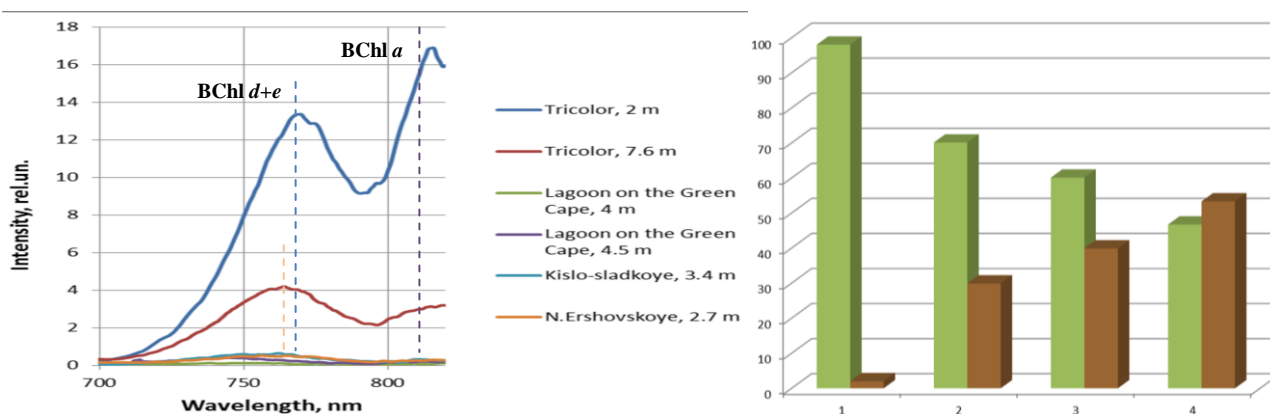


Figure 1: left – fluorescence emission spectra of water samples with green sulphur bacteria ($\lambda_{ex} = 440$ nm); right – ratio of different types of bacteria in water bodies. 1: *Trekhtzvetnoe* ("Tricolor"), 2 m depth; 2: *N. Ershovskoye*, 3.4 m depth; 3: *Kisló-Sladkoye*, 3.4 m depth; 4: *Lagoon on the Cape Zeleny* ("Lagoon on the Green Cape"), 4.5 m depth.

The research was targeted to measure the depth distribution of green- and brown-coloured green sulphur bacteria living in the several natural reservoirs found in different stages of isolation from the sea (Kandalaksha Bay, White Sea). To perform such measurements in different seasons we conducted a spectral analysis of natural water samples containing microorganisms during expeditions carried out in February 2015 and March 2016. Samples of water with microorganisms were

collected with a submersible pump, and the optical density spectra of the samples, and of some monocultures of green- and brown-coloured sulphur bacteria cultivated in the laboratory, were measured with a Unico spectrophotometer. Fluorescence emission and excitation spectra were recorded with a Solar CM2203 luminescence spectrometer. Using a specially developed spectral algorithm we identified the ratio of different types of bacteria at various depths in all the studied water bodies and measured the concentration of bacteriochlorophylls in bacterial cells (3). Using an optical microscope, a hemocytometer, and the concentration data we estimated the content of bacteriochlorophylls in each type of bacteria. About 250,000 molecules bacteriochlorophyll *d* were found in one cell of green-coloured sulphur bacteria, in the cell of brown-coloured bacteria were detected 73,000 molecules of bacteriochlorophyll *e*.

REFERENCES

- 1 Krasnova E D, A V Kharcheva, I A Milutina, D A Voronov & S V Patsaeva, 2015. Study of microbial communities in redox zone of meromictic lakes isolated from the White Sea using spectral and molecular methods. Journal of the Marine Biological Association of the United Kingdom, Special Section: European Marine Biology Symposium Papers 2014, 95(8): 1579-1590. DOI: 10.1017/S0025315415000582
- 2 Proceedings of Winogradsky Institute of Microbiology. Vol. 15: Photosynthetic Microorganisms, edited by V F Galchenko. MAKS Press, 2010, 352 pp.
- 3 Kharcheva A, A Zhiltsova, O Lunina, A Savvichev & S Patsaeva, 2016. Quantification of two forms of green sulfur bacteria in their natural habitat using bacteriochlorophyll fluorescence spectra. Proceedings of SPIE, 9917: 99170P-1-99170P-82