SPECTRAL STUDY OF ANOXYGENIC PHOTOTROPHIC MICROORGANISMS IN SEVERAL STRATIFIED LAKES AT THE KANDALAKSHA BAY OF THE WHITE SEA

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The natural reservoirs found at different stages of isolation from the White Sea are a special group of hydrological objects. Their origin and evolution are associated with the uplift of the Kandalaksha Bay coast with the speed of about 4 mm per year (1). Stratified structure in such water bodies is formed under the influence of conditions affecting not only physicochemical characteristics of the water but biological ones as well.

In aquatic systems with abnormal circulation of water the anoxygenic phototrophic microorganisms, green sulfur bacteria (GSB), may be of a particular interest. These bacteria are widespread in such water bodies because they are able to use hydrogen sulfide instead of water in photosynthesis. Photosynthetic pigments of GSB are bacteriochlorophylls (BChls) and carotenoid pigments. GSB are divided into two types according to their pigmentation: green-coloured bacteria containing BChls *c* and *d* and carotenoid chlorobactene, and brown-coloured bacteria containing BChl *e* and carotenoid isorenieratene. The presence of the certain type of bacteria in environment depends on several conditions and may vary depending on the status of the reservoir and external factors (2,3). The optical properties of the pigments make possible the environmental monitoring and the study of the properties of microorganisms *in situ* using spectral methods.

In this work the spectral properties of pure cultures of GSB were explored. The optical density spectra were measured with the Solar PB 2201 spectrophotometer, and the fluorescence emission and excitation spectra were recorded by the Solar CM2203 luminescence spectrometer. Absorption and fluorescence bands demonstrate the differences between two types of bacteria (Fig.1). The typical wavelengths of absorbance peaks for green-colored bacteria are 455-469, 518-526, 716-724. Absorbance peaks for brown-colored bacteria are located at the wavelengths 426-428, 442-448, 719-728 nm. A minor absorption peak at 800 nm which arises from the BChl *a* was observable both for green-colored and brown-colored bacteria.

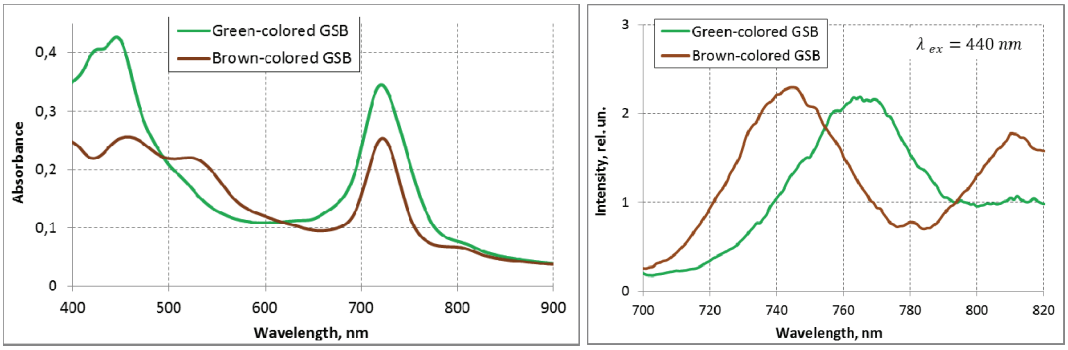

Figure 1: Absorption (left) and fluorescence (right) spectra of pure cultures of green sulfur bacteria.

According to these facts BChl *c* does not contribute to the spectral properties of pure cultures noticeable. The major photosynthetic pigments in the cell of green-colored and brown-colored bacteria are BChl *d* and BChl *e* respectively. The wavelength of fluorescence maximum is located typically within 758 - 766 nm for BChl *d* and 743 - 747 nm for BChl *e*. The optimal wavelength of fluorescence excitation for all photosynthetic pigments presented in bacteria (BChl *d*, *e* and *a*) was found as 440 nm (spectral slit 5 nm).

After analysis of emission fluorescence spectra excited at 440 nm for many cultures we developed (4) and improved spectral method to found out the ratio of different types of bacteria in water sample. This algorithm includes fitting of the fluorescence spectrum by Gaussian curves for each of BChl type. We found parameters of approximating curves for different types of bacteria: the fluorescence peak in the 740-820 nm area for BChl *d* approximates the Gaussian curve with λ_{max} = 760 nm and width w_g = (56,9 ± 1,4) nm. For brown-colored bacteria λ_{max} = 745 nm and width w_b = (46,5 ± 0,5) nm (Fig. 2). These results are important for spectroscopy of highly aggregated molecules as well as for further *in situ* detection of two types of bacteria.

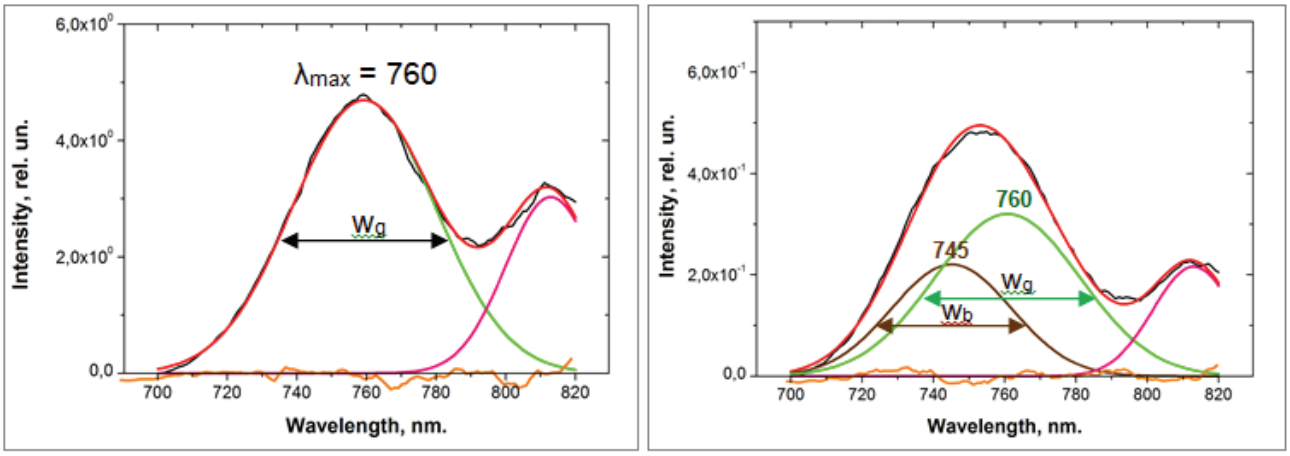

Figure 2: The fitting of fluorescence band by Gaussian functions for green-colored bacteria (left) and for the natural water sample of the lake Bolshie Khruslomeny (right).

The fluorescence bands of bacterial cells containing BChl *e* are narrower than cells having the photosynthetic pigment BChl *d*. This fact indicates a smaller amount of the pigment molecules in light-harvesting complex of brown-colored type of bacteria in comparison to green-colored GSB. The result was confirmed using an optical microscope, a hemocytometer, and the data of the concentration of the cells. We estimated the content of BChls in each type of bacteria. One cell of green-coloured sulfur bacteria contains about 250,000 molecules BChl *d*, and the cell of brown-coloured bacteria - 73,000 molecules of BChl *e*.

The analysis of natural water samples from several water bodies was conducted using the developed methods (Fig. 2, right). During the expedition in March 2017 water was sampled at various horizons from several relic lakes: Trekhtzvetnoe (“Tricolor”), N. Ershovskoye, Lagoon on the Cape Zeleny ("Lagoon on the Green Cape") and Bolshie Khruslomeny (“Large Hruslomeny”). Absorption and fluorescence measurements performed in water samples from different depths in laboratory allowed to plot depth distribution of anoxygenic phototrophic microorganisms and to calculate partial concentrations of two types of GSB at different depths in those lakes.

ACKNOWLEDGEMENT

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