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OPTICS AND SPECTROSCOPY. LASER PHYSICS

Bacteriochlorophyll Fluorescence of Green Sulfur Bacteria in Anaerobic Zone of Two Natural Water Bodies

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Abstract—Absorption and fluorescence spectra for living cells of green sulfur bacteria inhabiting the anaerobic zone of two meromictic lakes separated from the White Sea have been studied. The spectral-optical properties of pure cultures of green-colored and brown-colored species of green sulfur bacteria *Chlorobium phaeovibrioides* have been compared, and the content of bacteriochlorophyll molecules in one bacterial cell of each species has been estimated. The method of separating the contributions of different groups of green sulfuric bacteria to bacteriochlorophyll fluorescence was applied for a mixture of two species of bacteria with different pigmentation. The depth distributions of fluorescence intensity and concentration of bacteriochlorophylls for microorganisms inhabiting the Trekhtzvetnoe and Elovoe lakes at the Kandalaksha Gulf of the White Sea were plotted.

Keywords: fluorescence spectra, absorption spectra, bacteriochlorophyll, green sulfur bacteria, *Chlorobium phaeovibrioides*.

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INTRODUCTION

Fluorescence of chlorophyll (Chl) is widely used for mapping the phytoplankton distribution in water reservoirs [1, 2], monitoring the effects of environmental stress on higher plants [3, 4], and biodiagnostics of the water environment [5, 6]. However, the spectral characteristics of bacteriochlorophylls (BChl), which are photosynthetic pigments of anoxygenic phototrophic bacteria, have been poorly studied [7]. Only a few works have been devoted to monitoring of phototrophic bacteria in the environment [8, 9].

Green sulfur bacteria are anoxygenic phototrophic microorganisms that inhabit the chemocline area in the transition between aerobic and anaerobic conditions and in the anaerobic zone of meromictic water reservoirs that have been separated from the White Sea [10–12]. These microorganisms use hydrogen sulfide from the bottom water layer of meromictic lakes or other compounds, such as molecular sulfur and molecular hydrogen, as electron donors. Green sulfur bacteria live at depths with low light intensity [13–16] and have specific antenna structures (chlorosomes) that contain a huge amount of BChl (up to 250 000 molecules) [17]. Morphologically similar bacterial species can differ in pigments of photosynthetic apparatus of cells. Thus, some of them are green-colored (BChl *c* and *d* and carotenoid chlorobactene) and others are brown-colored (BChl *e* and carotenoid isorenieratene) [18, 19]. Chlorobactin and BChl *c*, *d*, and *e* pigments of the green-colored green sulfur bacteria absorb blue and red light. Isorenieratine of the brown-colored green sulfur bacteria absorbs light at the wavelength of 500–550 nm. Therefore, brown strains predominate at considerable depths and only green light permeates at these depths [20, 21]. Both species of sulfur bacteria also contain BChl *a*, which functions in reaction centers and antenna, and small amounts of Chl *a* in reaction centers [22]. Closely located and mutually oriented BChl molecules that compose the antenna provide the directed energy migration of high efficiency [23].

Two main absorption bands are typical for the absorption spectra of BChl *c*, *d*, and *e*: in the blue zone of the spectrum and a wavelength maximum in the far red zone [24, 25]. The wavelength maxima of the absorption spectra in intact cells of green sulfur bacte-

ria are located in the range of 745–755 nm (BChl *c*), 715–745 nm (BChl *d*), and 710–725 nm (BChl *e*) [26]. It is noteworthy that light absorption is carried out by large BChl aggregates, since BChl monomers in organic solvent, for instance, absorb light in the range of 600–700 nm [18].

Two main overlapping bands are characteristic for the fluorescence spectra of BChl in the cells of green sulfur bacteria. They have maxima in the range of 740–770 nm (BChl *c*, *d*, and *e*) and 810–815 nm (BChl *a*). Emission peaks can shift depending on the bacterial species and their pigments. Brown forms containing BChl *e* in their composition have a maximum at the wavelength of 740 nm [27]. A peak at the wavelength of 770 nm is typical for the green forms containing BChl *c* and *d* [26]. The second fluorescence maximum corresponds to light emission by BChl *a* molecules. Two forms of anoxygenic bacteria often coexist at the same depths in water reservoirs. Therefore, when express analysis of natural water containing green sulfur bacteria is used, peaks for BChl *c*, *d*, and *e* are often fused into one band. The emission wavelength at the maximum of the enveloping band depends on the ratio of the concentrations of different species of bacteria. Therefore, the relative contribution of two groups of bacteria can be estimated according to the position of this maximum by division of the spectrum into two components of Gaussian form at the wavelength maxima of 740 and 770 nm [14].

1. MATERIALS AND METHODS

Samples of natural water from Lake Trekhtsvetnoe and Lake Elovoe, at different stages of isolation from the White Sea, were collected in July 2016 from different horizons with a submersible pump at 50-cm intervals and in the zone of the chemocline using a multisyringe sampler with an interval of 2.5 cm.

Monocultures of green-colored *Chlorobium phaeovibrioides* (density of cell suspension $C = 5.8 \times 10^7$ cells/mL), brown-colored bacteria *Ch. phaeovibrioides* ($C = 3.1 \times 10^7$ cells/mL), and their mixtures in different ratios were used to determine spectrofluorescence characteristics of BChl in different types of sulfur bacteria. Cell counts were performed in a Goryaev chamber under a microscope using the standard technique. Acetone–methanol (7 : 2) extracts were prepared to determine BChl concentration in cultures and samples of natural water, according to the standard method [28, 29].

Fluorescence spectra of green sulfur bacteria and water samples were measured with a Solar CM2203 fluorometer at the excitation wavelength $\lambda_{ex} = 440$ nm. The absorption spectra of culture extracts and water samples were registered with a Unico spectrophotometer in quartz cuvettes with an optical path length of 1 cm.

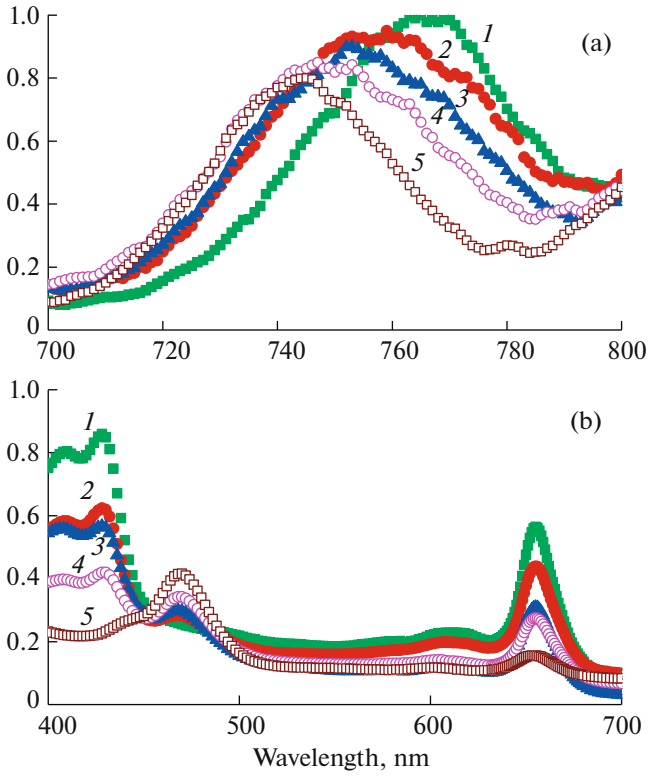

Fig. 1. The dependence of the light emission maximum position of bacteriochlorophyll in the fluorescence spectra on the concentration ratio of the two types of green sulfur bacteria (a) and changes in the absorption bands in the spectra of optical density of extracts at different concentration ratios of the two types of green sulfur bacteria *Chlorobium phaeovibrioides* (b). Only green-colored cultures (*1*); 75% green-colored and 25% brown-colored cultures (*2*); 60% green-colored and 40% brown-colored cultures (*3*); 45% green-colored and 55% brown-colored cultures (*4*); only brown-colored cultures (*5*).

2. RESULTS AND DISCUSSION

2.1. Spectral Studies of Green Sulfur Bacteria Monocultures

A fluorescence peak at 740–770 nm was found in the fluorescence emission spectra of the monocultures of green sulfur bacteria and their mixtures. The peak corresponded to light emission by BChl *c*, *d*, and *e* molecules (Fig. 1a). Mixtures with different percentage ratios of the monocultures of the two types of bacteria were prepared to determine the position of this maximum depending on the concentration ratios of different bacterial species. These were (*1*) only green-colored cultures; (*2*) 75% green-colored and 25% brown cultures (of the sample volume); (*3*) 60% green-colored and 40% brown-colored cultures; (*4*) 45% green-colored and 55% brown-colored cultures; and (*5*) only brown-colored cultures. The fluorescence band of BChl *c*, *d*, and *e* was approximated by two components of Gaussian form with wavelength maxima at 745 and 765 nm. The calculated S_{765}/S_{745}

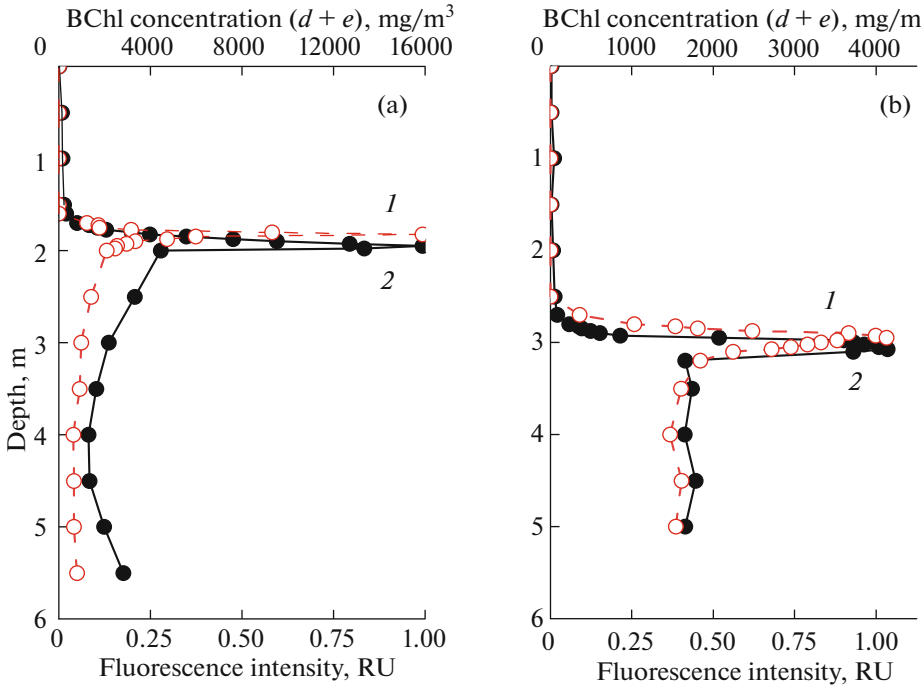

Fig. 2. The vertical distribution for the relative fluorescence intensity of bacteriochlorophylls in green sulfur bacteria (*1*) and concentrations of bacteriochlorophylls ($d + e$) (*2*) in Lake Trekhtsvetnoe (a) and Lake Elovoe (b) in July 2016.

area ratio below the corresponding curves agreed well with the percentage ratios of concentrations of two bacterial species in the sample volume. The proposed method can be used to separate the contribution of diverse bacterial species in the samples of natural water.

Acetone–methanol extracts were prepared and their optical density spectra in the spectral range of 200–1000 nm relative to the acetone and methanol solutions (7 : 2) were registered for quantitative determination of the BChl content in the cultures of green sulfur bacteria (Fig. 1b). Application of the method of bacterial cell counts under a microscope and calculation of the BChl concentration by extract absorption spectroscopy [28, 29] made it possible to calculate the average content of BChl molecules per one bacterial cell. The green-colored green sulfur bacterium contained 25.0×10^4 molecules of BChl *d*, while the brown-colored contained 7.3×10^4 molecules of BChl *e*.

2.2. Spectral Studies of Natural Water Samples

Three water layers, which are fundamentally different in their spectralfluorescence and physicochemical characteristics, compose the studied lakes [30, 31]. These are a transparent surface layer with oxygenic phototrophic microorganisms (algae and cyanobacteria); a strongly colored (from emerald-green to grayish-brown) layer with high concentration of green sulfur bacteria in the zone of chemocline, which corresponds to the change of the aerobic zone to the anaerobic zone [32]; and a bottom layer with sedimentating and moribund green sulfur bacteria, hydrogen sulfide at a high concentration, and poor intensity of illumination [14]. According to the literature data [31, 33], the upper layer in the lakes is gradually desalinated as a result of the inflow of swamp and rain water, the chemocline shifts to deeper water, while the physicochemical characteristics of the water in the bottom part of lakes remain almost unchanged.

Similarly to the description in Section 2.1, the fluorescence spectra in water samples from Lake Trekhtsvetnoe and Lake Elovoe were measured. The vertical distributions of the relative fluorescence intensity of BChl in green sulfur bacteria were plotted according to the fluorescence of natural water in the range of 740–770 nm. BChl $d + e$ concentrations were calculated via the absorption spectra of acetone–methanol extracts and the vertical distributions of the contents of these photosynthetic pigments in Lake Trekhtsvetnoe and Lake Elovoe were plotted.

The layer with the highest content of green sulfur bacteria was as thick as 30 cm in Lake Trekhtsvetnoe; it was located at a depth of 1.7–2.0 m (Fig. 2a). The maximum concentration of green sulfur bacteria was detected at the depth of 1.825 m; the BChl $d + e$ concentration was 15.9 ± 0.9 g/m^3.

It is noteworthy that the depth distribution of the fluorescence intensity of BChl does not agree with the

distribution of the concentrations of these pigments, which was determined by the absorption spectra of water sample extracts. The highest value of BChl fluorescence intensity was located at the depth of 1.900 m, i.e., 7.75 cm lower than the peak of pigment concentration. We suggest that this results from the suppression of BChl fluorescence at these depths, since impairment of the process of excitation energy transfer in the photosynthetic apparatus is known to occur in the cells of green sulfur bacteria at low values of the redox potential (Eh) [34, 35] and in the presence of oxygen [36]. The number of bacterial cells and pigment concentration abruptly decreased at the depth of 2.1 m. The fluorescence intensity below the chemocline also gradually decreased towards the bottom and remained almost the same in the entire underlying water mass.

Green sulfur bacteria occurred in Lake Elovoe at the depth of 2.7 m (Fig. 2b). Their concentration increased abruptly with an increase in depth to 2.95 m, while the maximum concentration of BChl was 4.13 ± 0.12 g/m^3. This was followed by a sharp decrease in the concentration of bacteria, which was, however, more gradual than in Lake Trekhtsvetnoe. Starting from the depth of 3.5 m and below, the concentration of green sulfur bacteria almost did not change. BChl fluorescence was detected at the depth of 2.7 m, in the same zone, where previously mentioned concentration of BChl in green sulfur bacteria was found. Fluorescence increased abruptly in the region of the highest concentration of bacteria at the depth of 2.9 m. However, it did not decrease further towards the bottom; on the contrary, it continued to increase, reaching a maximum at the depth of 3.075 m, i.e., by approximately 10 cm lower than the horizon containing the highest number of green sulfur bacteria, which was similar to Lake Trekhtsvetnoe. Directly below the layer with the maximum fluorescence, the intensity decreased sharply and remained low in the depth range of 3.2–5.0 m.

CONCLUSIONS

Anoxygenic phototrophic microorganisms of the chemocline and anaerobic zone of two meromictic water reservoirs that separated from the White Sea (Lakes Trekhtsvetnoe and Elovoe) were studied with spectral methods using natural water containing bacteria from different horizons and isolated monocultures of *Ch. phaeovibrioides*. The depth distributions of green sulfur bacteria, including independent distributions for green and brown forms, were obtained. Our data indicate that the peak of bacterial abundance is at a more considerable depth in the reservoir containing the brown-colored strain as the predominant strain than in the reservoir with the prevailing green-colored strain. This agrees with the presence of an additional pigment, carotenoid isorenieratene, in the photosynthetic apparatus of the brown bacteria. This carotenoid absorbs light in the range of 500–550 nm, which deeply permeates the high-density microbial community of the meromictic water reservoir. The maximum of the depth distribution of BChl fluorescence intensity in bacterial cells in each of the lakes is located at the depth, which is approximately 8–10 cm below the horizon containing the highest concentration of pigments, measured according to the absorption spectra of water samples extracts. This may indicate the impairment of the process of excitation energy transfer between the BChl molecules of the photosynthetic apparatus of green sulfur bacteria in the zone that contains their maximum cell numbers. The number of BChl molecules per one bacterial cell was calculated for monocultures. The green-colored green sulfur bacterium contained 25.0×10^4 molecules of BChl *d*, while the brown-colored bacterium contained 7.3×10^4 molecules of BChl *e*.

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