CALCULATING THE COMPOSITION OF MIXED POPULATIONS OF GREEN SULFUR BACTERIA USING DECOMPOSITION OF BACTERIOCHLOROPHYLL ABSORBANCE SPECTRA INTO INDIVIDUAL COMPONENTS

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The green sulfur bacteria (Chlorobiaceae) are a family of obligatory anaerobic photoautotrophic bacteria. These bacteria in natural populations are presented in two different types according to their pigmentation: green-coloured and brown-coloured. The green-coloured green sulfur bacteria contain bacteriochlorophyll *d,* and therefore their absorption spectrum has peak at 430 nm, whereas the brown-coloured green sulfur bacteria contain bacteriochlorophyll *e* with absorption maximum located at 470 nm. This difference of absorption bands allows estimating the proportion of different types of green sulfur bacteria in a mixed bacterial population. Here we describe a developed method of decomposition of mixed absorption spectra into individual spectra which allows estimating the proportion of bacteria of both types, and we apply this approach to obtain depth distribution of bacteria with different pigmentation.

We studied green sulfur bacteria collected in March 2016 from the meromictic (stratified) lake Trekhtzvetnoe (“Tricolor”) near the Kandalaksha Bay of the White Sea Separated from the sea the lakes are unique ecosystems with a straight vertical distribution of physicochemical characteristics and microorganisms not alternating through the year. In the area of Kandalaksha Bay several lakes are known being on a different stage of separating from the sea (1,2). Lake Trekhtzvetnoe is on the last stage of isolation from the White Sea. This lake contains a layer of the bright green water with a specific anoxygenic photosynthetic microbial community, green sulfur bacteria.

Extracts were prepared from natural water samples collected at different depths, as well as from pure cultures cultivated in laboratory conditions, using an acetone-methanol (7:2) mixture. Absorption spectra of bacterial extracts were measured using a Unico spectrophotometer against the acetone-methanol mixture.

Typical absorption spectra of green sulfur bacteria extracts include two bands in the blue and red regions (3). To separate the distribution of bacterial cultures with different pigmentation the method of bacteriochlorophyll *d* and *e* concentration ratio was offered in this work, using calculation of the ratio of areas under these absorption peaks. Total concentration of bacteriochlorophyll *d*+*e* was calculated using an empirical formula from (4).

The vertical distribution of bacteriochlorophyll concentration shows that the highest content of green sulfur bacteria was located at 2.0 m depth in the chemocline area. About 93% of green sulfur bacteria were green-coloured and the remaining 7% were brown-coloured. The highest concentration of green-coloured green sulfur bacteria was located at 2.0 m depth (3500 mg/m^3) and with increasing depth their concentration evenly reduced. An essential content of brown-

coloured green sulfur bacteria appeared at 2.0 m depth (187 mg/m^3); their concentration increased to 3.0 m depth (250 mg/m^3) and then slowly decreased.

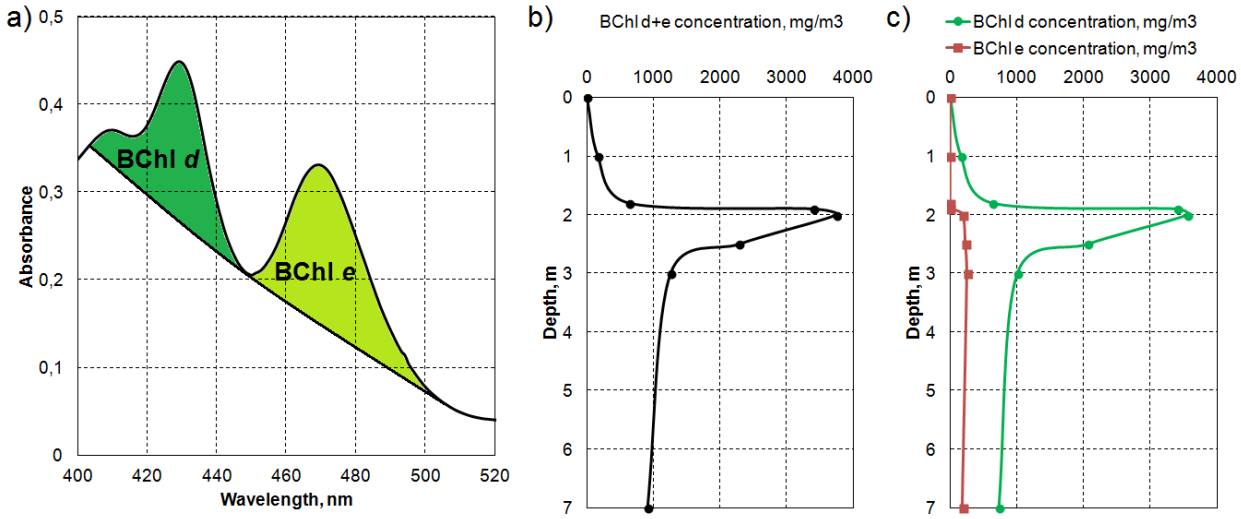

Figure 1: a) Absorbance spectrum of culture extract in acetone-methanol (mixture of green-coloured and brown-coloured cultures In proportion 1:1); b) vertical distribution of bacteriochlorophyll d+e concentration in lake Trekhtzvetnoe; c) vertical distribution of bacteriochlorophyll d and e concentrations in lake Trekhtzvetnoe calculated using the suggested method.

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REFERENCES

1 Krasnova E, A Pantyulin, T Belevich, D Voronov, N Demidenko, L Zhitina, L Ilyash, N Kokryatskaya, O Lunina, M Mardashova, A Prudkovsky, A Savvichev, A Filippov & V Shevchenko, 2013. Multidisciplinary Studies of the Separating Lakes at Different Stage of Isolation from the White Sea Performed in March 2012. Oceanology, 53(5): 714-717

2 Krasnova E, D Voronov, N Frolova, A Pantyulin & T Samsonov, 2015. Salt lakes separated from the White Sea. EARSeL eProceedings, 14(S1): 8-22

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-8

4 Lunina O, A Savvichev, B Kuznetsov, N Pimenov & V Gorlenko, 2013. Anoxygenic phototrophic bacteria of the Kislo-Sladkoe stratified lake (White Sea, Kandalaksha Bay). Microbiology, 82 (6): 815-832