Quantification of bacteriochlorophylls $d$ and $e$ in water with green sulfur bacteria: spectroscopic approaches

Optical characteristics of water in stratified lakes are important to study in connection with the observation of colored layers in their chemocline zone resulted from the massive development of anoxygenic phototrophic bacteria. Several water bodies on the coast of the Kandalaksha Bay of the White Sea are known as being at different stage of isolation from the Sea. The development of phototrophic green sulfur bacteria indicates the presence of hydrogen sulfide in deep water layers; therefore, it is important to have reliable and fast methods to assess concentration of phototrophic bacteria and their main pigments, bacteriochlorophylls (BChl). We describe several spectral techniques for measuring BChl $d$ and BChl $e$ in extracts in organic solvents with preliminary concentration of bacteria using filtration, as well as methods for measuring pigments directly in water with microbial cells.

Methods and materials:
The absorption spectra were measured with the Solar PB 2201 or Solar PV 1251 spectrophotometer, and the fluorescence emission and excitation spectra were recorded by the Solar CM2203 luminescence spectrometer. The extraction of bacteriochlorophylls in an organic solvent were prepared using a mixed solvent acetone-ethanol taken in a ratio of 7:2. Three spectral methods of BChl quantification were tested on natural water from several meromictic reservoirs of the Kandalaksha Bay of the White Sea, sampled in the expeditions performed in March and September 2021.

Method 1. The calculation of BChl concentration using absorption spectra of extracts was made according to the formula:

$$C(BChl\ d) = \frac{D_{655}}{\varepsilon_{BChl\ d}} \frac{V}{d} \times 10^4,$$

where $D_{655}$ is the absorbance of BChl $d$ at 655 nm; $\varepsilon$ is the volume of acetone-methanol extract [ml]; $V$ is the volume of the water sample [ml]; $d$ is the optical path length [cm]; $\varepsilon_{BChl\ d} = 98$ cm$^2$/mg is the extinction coefficient for BChl $d$.

Method 2. The calculation of BChl concentration using absorption spectra of natural water samples was made by the empirically deduced formula:

$$C(BChl\ d) = A \cdot \Sigma D,$$

where $\Sigma D$ is the absorbance peak area [nm]; $A$ is the coefficient of proportionality, $A = 336 \pm 9$ mg/(nm $\cdot$ m$^2$).

Method 3. The calculation of BChl concentration using fluorescence spectra of extracts was made using the assumption of the following proportionality:

$$C(BChl\ d) = F_{\text{max}} \cdot \frac{c_{\text{max}}}{F_{\text{max}}} \cdot \frac{F}{F_{\text{max}}} \cdot F_{\text{max}}$$

where $c_{\text{max}}$ is the maximum of recorded concentration in the series of samples [mg/m$^3$]; $F$ is the meaning of the fluorescence corresponding to this concentration [in relative units].

Major results: We obtained the concentration of BChl $d$ for water samples from different depths of lake Trekhtzvetnoe by all methods. The results were demonstrated on a bacterial plate for the first time. The obtained values are similar in value, it makes the methods suitable for practical use.

Conclusion: Method of the calculation of BChl concentration using absorption spectra of extracts is the most sensitive method in cases of low concentration. It is suitable to register concentration less than 360 mg/m$^3$. However it requires special sample preparation – the extraction of pigments. For this reason one of the advantages of the calculation of BChl concentration using absorption spectra of natural water samples is a high speed of processing. We have invented a new method of the calculation of the pigment concentration using fluorescence spectra. This allowed us to establish a connection between the concentration of the pigment and its fluorescent properties. The obtained results can be useful for future work.

The work was performed using the financial support of the RFBR grant No. 19-05-00377 and the Theoretical Physics and Mathematica Advancement Foundation “BASIS”.