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Extinction coefficients of bacteriochlorophylls *d* and *e* in organic solvents for quantitative spectrophotometric determination of pigments of phototrophic green sulphur bacteria

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The extinction coefficients of bacteriochlorophylls *d* and *e* in several organic solvents were determined and compared with the coefficients of related compounds - chlorophylls *a* and *b*. In some solvents (ethanol, isopropanol, acetone-ethanol (7:2) and acetone-methanol (7:2) — for bacteriochlorophyll *d*, in isopropanol and acetone-ethanol (7:2) — for bacteriochlorophyll *e*), extinction coefficients were determined for the first time. Spectrophotometric formulas for quantitative measurement of bacteriochlorophylls *d* and *e* in different solvents are given. The method of dynamic light scattering revealed the absence of noticeable aggregation of bacteriochlorophyll molecules in acetone and ethanol to a concentration of 0.14 g/l. The obtained data expand the possibilities of the spectrophotometric method for quantifying the pigments of phototrophic bacteria.

Keywords: bacteriochlorophyll, absorption spectra, extinction coefficients, organic solvents, anoxygenic phototrophic bacteria.

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Introduction

Owing to such advantages as rapidity, efficiency, and capacity for analysis of materials in large quantities, optical methods for determination of photosynthetic pigments of microorganisms are widely adopted in certain practical applications, which include the study of water systems. These studies are focused mostly on the determination of chlorophyll (Chl) concentration in algal or cyanobacterial cells [1–5]. However, in some cases, in addition to Chl, researchers are interested in the determination of photosynthetic pigments of phototrophic bacteria, bacteriochlorophylls (BChl). BChl molecules are tetrapyrrole pigments of anoxygenic phototrophic bacteria and perform photosynthesis without the release of oxygen. Various types of BChl molecules are known. BChl *a* is the dominant chlorin pigment in reaction centers of most phototrophic proteobacteria and all green sulfur bacteria (*Chlorobiaceae*) and filamentous anoxygenic phototrophs (*Chloroflexia*). In a few phototrophic proteobacteria, BChl *a* is substituted completely with BChl *b*. Bacteriochlorophyll *g* was found only in a single group of bacteria (heliobacteria) that comprises a small number of rare species. The so-called chlorosome BChls (BChls *c*, *d*, *e*, *f*) are found exclusively in special photosynthetic antenna complexes (chlorosomes) that are present in all green sulfur bacteria (*Chlorobiales*), several filamentous anoxygenic phototrophs (*Chloroflexia*), and one recently discovered photoheterotrophic acidobacterium (*Chloracidobacterium thermophilum*) [6–8].

The optical properties of BChl *b* and one chlorosome BChl, BChl *c*, have already been examined in [9–11] and [12–16], respectively. However, the spectral properties of other BChls remain understudied. Molecules of BChls *c*, *d*, and *e* are similar to Chl in featuring a chlorin macrocyclic ring with lateral substituents that form the characteristic spectral features of these compounds [17]. Contrary to their name, chlorosome BChls (BChls *c*, *d*, and *e*) are closer in their chemical structure to Chl *a* and *b* than to other BChls (BChls *a*, *b*, and *e*). Chlorosome BChls and Chls *a* and *b* belong to the same chemical group of metalloporphyrins (namely, Mg chlorins), while BChls *a*, *b*, and *e* are Mg bacteriochlorins [11]. In contrast to all other Chls and BChls, BChls *c*, *d*, and *e* lack the $-COOCH_3$ substituent and have the $-CHOH-CH_3$ substituent. This enables their aggregation into certain macromolecular structures and ensures dense packing of BChl molecules in chlorosomes [18].

Absorption spectra of BChl in samples of natural water with microorganisms and in extracts are used to estimate the BChl concentration in water bodies [19–24]. BChls in chlorosomes of green sulfur bacteria cells differ from those in solutions in being highly aggregated, this leads to a difference in spectral properties manifested as a significant red shift of the long-wavelength absorption band of BChl after aggregation [25]. When extracting BChl from bacterial

cells, one has to work with organic solvents, which is an integral part of any spectral and chromatographic studies. To measure the concentration of pigments one needs to know the extinction coefficients of a given pigment in a specific solvent, which is used for extraction of this pigment from cells of phototrophic organisms.

The extinction coefficient of BChl is a physical quantity that characterizes the absorption of light by matter at a given wavelength and depends on the BChl type and the solvent used. According to the Bouguer–Beer–Lambert law, monochromatic light with a certain wavelength passing through an optically thin layer of matter is attenuated in proportion to the layer thickness and the concentration of a substance absorbing this light. In a layer of finite thickness, attenuation follows an exponential law. The extinction coefficients of BChl *d* in acetone and methanol solutions and the extinction coefficients of BChl *e* in acetone, methanol, ethanol, and 7:2 acetone-methanol solutions [26,27] are already known. If one uses the extinction coefficient of BChl *d* in a certain solvent instead of the coefficient for BChl *e* in the same solvent, the error of spectrophotometric determination of the pigment concentration may reach 50% [28]. The lack of complete data on the absorption characteristics of chlorosome BChls (BChls *d* and *e*) in such solvents as, e.g., a 7:2 mixture of acetone and methanol, which is used widely for chromatographic isolation of pigments [27,29–32], makes the problem of determination of BChl extinction coefficients important and relevant.

The efficiency of extraction of lipophilic pigments like molecules of BChl depends on several factors: solvent polarity [33], type of species of organisms, location and orientation of the intracellular pigment, and interaction between the solvent and the membrane [34]. As a result, it is (as of now) impossible to pick one solvent that would be fully suitable for extraction of photosynthetic pigments from cells of microorganisms. The dependence of spectral properties of BChl on the type of pure or mixed solvents implies that its extinction coefficients in them differ, necessitating the identification of correct extinction coefficients for proper interpretation of spectrophotometric data.

The data obtained in the present study should help refine spectrophotometric methods for determination of photosynthetic pigments of anoxygenic phototrophic bacteria. This line of research is highly relevant to monitoring of stratified water bodies with sulfide anoxia (relict water bodies in the Arctic included), which may develop naturally or under the influence of anthropogenic factors [35–37].

1. Objects and methods of study

Water samples with two forms of green sulfur bacteria (GSB) *Chlorobium phaeovibrioides* (*Chlorobiaceae*) were examined. Green sulfur bacteria are phototrophic microorganisms that carry out anoxygenic photosynthesis using hydrogen sulfide as an electron donor. Green sulfur bacteria are found in the anaerobic zone of water bodies which receives sunlight from the surface and contains reduced sulfur compounds. Such conditions are established, e.g., in the chemocline region of water bodies that get separated from the White Sea as a result of gradual elevation of the coast [35,36]. Two forms of GSB with different pigmentation (green GSB with BChl *d* and the chlorobactene carotenoid and brown GSB, which use BChl *e* and the isorenieratene carotenoid as photosynthetic pigments) were studied. A submerged pump was used to sample natural water from the chemocline of two separating lakes in the Kandalaksha Gulf of the White Sea in expeditions undertaken in 2021 and 2022. Water with green GSB was sampled from Lake Trekhtzvetnoe at the depth of 2.2 m, while water with brown GSB was sampled from a lagoon at Zelenyi Cape at the depth of 5.3 m. The choice of depth was determined by the location of the zone with the maximum concentration of microorganisms in the water column. Only one form of GSB was present in the chemocline of each of these water bodies. This was verified by the results of bacterial genome sequencing [38,39] and allowed us to use different water samples as sources of a specific BChl. The purity of pigments found in natural water was checked by examining the fluorescence emission spectra, since their parameters (peak wavelength and width of the fluorescence band) vary with the type of BChl present in samples [40]. Microorganisms from the lakes were concentrated on glass fiber filters with a pore size of 2μm with the use of a vacuum filtration unit. Dried samples were used to prepare concentrated extracts of pigments in acetone.

1.1. Preparation of extracts

Four organic solvents (acetone, methanol, ethanol, and isopropanol) and two prepared mixtures (a 7:2 acetone-ethanol solution and a 7:2 acetone-methanol solution) were used to prepare BChl solutions for spectral measurements. In order to eliminate the influence of solvents on the process of pigment release from bacterial cells into a solution, two concentrated extracts of BChls *d* and *e* in acetone were prepared in advance and subsequently diluted 50 times with each of the above-mentioned solvents. The samples obtained after this dilution are referred to as „base“ solutions. Thus, the concentration of BChls *d* or *e* in the monomeric form was the same in each „base“ extracts. In order to prepare extracts with different concentrations, each of the 12 „base“ extracts was diluted with the corresponding solvent in such a way as to obtain 50%, 25%, and 15% solutions of BChl *d* or *e* relative to the „base“ solution. In subsequent experiments, the proportionality of absorbance of a diluted solution to the concentration of a pigment in this solution was verified for each solvent.

1.2. Spectral measurements

Absorbance spectra of extracts with various concentrations of BChl *d* or *e* were measured using a Solar PB2201

spectrophotometer against to a pure solvent within the 300–800 nm spectral range with a step of 1 nm. Standard quartz cuvettes with an optical path length of 1 cm were used for measurements. Prior to measurements, extracts were centrifuged in an Elmi CM-50 centrifuge at 13000 rpm for 10 min in order for suspended particles in solutions to settle and for excess scattering in spectra to subside. The obtained spectra were not subjected to mathematical processing (smoothing or normalizing).

1.3. Examination of aggregation of bacteriochlorophylls in organic solvents

The potential for aggregation was examined in each series of diluted solutions and in the „base" extract: the proportionality of absorbance of a solution to the concentration of a pigment in this solution and the retention of shape of absorption spectra after dilution of the solutions were verified. Aggregation processes were not revealed; the shape of spectra after normalization to absorption at the maximum was retained.

The particle size in solutions with the highest concentration in acetone and ethanol (0.14 g/l for BChl *d* and 0.09 g/l for BChl *e*) was determined by dynamic light scattering with the use of a Photocor particle size laser analyzer. No particles possibly representing BChl aggregates were found in concentrated solutions in acetone or ethanol. The absorption spectra of concentrated solutions of BChls *d* and *e* in acetone and ethanol measured in thin cuvettes (0.5 cm) had the same shape as the absorption spectra of more dilute solutions in the corresponding solvents. Therefore, we assume that BChl *d* and *e* molecules do not form aggregates within the entire studied range of concentrations (up to 0.14 g/l).

1.4. Spectrophotometric method for determination of the concentration of bacteriochlorophylls in extracts

According to the Bouguer–Beer–Lambert law, absorbance D, which is defined as the decimal logarithm of the ratio between the intensity of light incident on the sample and the intensity of light emerging from the sample, is proportional to the concentration of absorbing molecules. The following is true for a BChl extract with concentration C [mol/l] and optical path length l [cm]:

$$D = \varepsilon_{10} C l, \tag{1}$$

where ε_{10} is a coefficient of proportionality that is referred to as the decimal molar extinction coefficient [l/(mol·cm)]. This decimal molar extinction coefficient is related to the molar one in the following way: $\varepsilon_{10} \approx 0.4343\varepsilon_{\text{mol}}$. Depending on the dimension of concentration C (expressed in g/l or mol/l), specific ε and molar ε_{mol} extinction coefficients are distinguished. They are related in the following way:

$$\varepsilon M = \varepsilon_{\text{mol}}, \tag{2}$$

where M is the molar mass of a pigment (BChl) [41].

In the transition to specific extinction coefficient ε and the concentration with the dimension of [g/l], we find the final formula that was used in the present study for calculating the concentration of BChl *d* or *e* in acetone:

$$C = D/0.4343\varepsilon l, \tag{3}$$

where D is the absorbance of an extract at a wavelength of 655 nm, l is the optical path length (1 cm), and ε is the extinction coefficient in acetone (98 l/g·cm for BChl *d* [26] and 58.6 l/g·cm for BChl *e* [27]).

Known extinction coefficients of BChls *d* and *e* are presented in Table 1. The used type of organic solvent, wavelength $\lambda_{\max}$ corresponding to the maximum absorbance, and the extinction coefficients of related compounds (Chls *a* and *b*) are also listed in this table. The missing values of molar or specific extinction coefficients were calculated in accordance with formula (2). The following literature values of molar masses of pigments needed for this calculation were used: M (BChl *d*) = 806.6 g/mol [42], M (BChl *e*) = 834.5 g/mol [27], M (Chl *a*) = 893.5 g/mol, and M (Chl *b*) = 907.5 g/mol [41].

1.5. Relative calculation of extinction coefficients of BChls *d* and *e* in various solvents

According to the Bouguer–Beer–Lambert law, the absorbances of two different solutions with the same pigment concentration measured in identical cuvettes (i.e., with the same optical path length) are specified exclusively by the values of extinction coefficients of a pigment in the used solvents. Let the extinction coefficient and the slope coefficient of the concentration dependence of absorbance maxima be equal to ε_1 and k_1, respectively, in solvent 1 and ε_2 and k_2 in solvent 2. The following is true due to the equality of pigment concentrations and optical path lengths:

$$\frac{k_1}{\varepsilon_1} = \frac{k_2}{\varepsilon_2}.$$

Or

$$\varepsilon_1 = \varepsilon_2 \frac{k_1}{k_2}.$$

Thus, if the extinction coefficient for a pigment in solvent 1 and the slope coefficients of concentration dependences of absorbance maxima in solvents 1 and 2 are known, one may determine the extinction coefficient for this pigment in solvent 2.

The errors for slope coefficients were calculated using the least-squares method, while the error for the extinction coefficient was determined as a standard deviation:

$$\sigma_{(\varepsilon_1)} = \sqrt{\left(\frac{\partial \varepsilon_1}{\partial k_1}\right)^2 S_{k_1}^2 + \left(\frac{\partial \varepsilon_2}{\partial k_2}\right)^2 S_{k_2}^2}$$

$$= \sqrt{\left(\frac{\varepsilon_2}{k_2}\right)^2 S_{k_1}^2 + \left(-\frac{\varepsilon_2 k_1}{k_2^2}\right)^2 S_{k_2}^2},$$

Table 1. Extinction coefficients of BChls *d* and *e* and Chls *a* and *b* (determined at the indicated wavelengths); values calculated using formula (2) are written in bold

Pigment	Solvent	λ_{max}, nm	ε, $l \cdot g^{-1} \cdot cm^{-1}$	ε_{mol}, $l \cdot mmol^{-1} \cdot cm^{-1}$	Reference
BChl *d*	Acetone	654	98.0	**79.0** (654 nm)	[26]
	Methanol	659	82.3	**66.4** (659 nm)	[26]
BChl *e*	Acetone	649	58.6	48.9 (649 nm)	[27]
	Acetone-methanol (7:2)	651	**49.6**	41.4 (651 nm)	[27]
	Ethanol	654	**49.0**	40.9 (654 nm)	[27]
	Methanol	660	**42.5**	35.5 (660 nm)	[27]
Chl *a*	Acetone	662	**88.2**	78.8 (662 nm)	[43]
	Ethanol (96%)	665	83.4	74.5 (665 nm)	[44]
	Methanol	665	**79.9**	71.4 (665 nm)	[45]
Chl *b*	Acetone	646	**51.3**	46.6 (646 nm)	[43]
	Ethanol (96%)	650	44.2	40.1 (650 nm)	[44]
	Methanol	652	**42.5**	38.6 (652 nm)	[45]

Table 2. Experimentally determined extinction coefficients of BChl ε_{exp} (at the indicated wavelength), corresponding slope coefficients k_i of the dependence of absorbance maxima on concentration in the solvent, and tabular extinction coefficients ε_{tab} taken from literature

BChl	Solvent	k_i, l/g	ε_{exp}, l/g·cm	ε_{tab}, l/g·cm
BChl *d*	Acetone	41.3 ± 0.3	**98** (655 nm)	**98** (654 nm) [26]
	Acetone-ethanol (7:2)	34.7 ± 0.3	82.5 ± 0.8 (657 nm)	–
	Acetone-methanol (7:2)	32.5 ± 0.3	77.3 ± 0.8 (657 nm)	–
	Isopropanol	35.2 ± 0.3	83.6 ± 0.9 (659 nm)	–
	Ethanol	34.2 ± 0.3	81.3 ± 0.8 (660 nm)	–
	Methanol	34.3 ± 0.3	81.5 ± 0.8 (661 nm)	82.3 (659 nm) [26]
BChl *e*	Acetone	25.8 ± 0.3	**58.6** (655 nm)	**58.6** (649 nm) [27]
	Acetone-ethanol (7:2)	23.8 ± 0.3	54.0 ± 1.1 (656 nm)	–
	Acetone-methanol (7:2)	24.6 ± 0.3	55.9 ± 1.1 (657 nm)	49.6 (651 nm) [27]
	Isopropanol	24.8 ± 0.3	56.2 ± 1.1 (657 nm)	–
	Ethanol	20.8 ± 0.3	47.3 ± 0.9 (659 nm)	49 (654 nm) [27]
	Methanol	21.4 ± 0.3	48.6 ± 0.9 (661 nm)	42.5 (660 nm) [27]

where S_{k_1} and S_{k_2} are the errors of slope coefficients k_1 and k_2, respectively.

2. Experimental results

2.1. Absorption spectra of extracts of BChls *d* and *e* in different solvents

All spectra of BChl extracts have two clearly distinguished absorption bands in the short- and long-wavelength regions (Fig. 1). The short-wavelength band of the green GSB form (with BChl *d* being the primary photosynthetic pigment) consists of two overlapping bands with absorbance peaks in the region of 412–415 and 430–433 nm (depending on the solvent). This corresponds to the absorption of light by BChl *d* and carotenoids (with chlorobactene being the primary one) [46]. A well-pronounced absorption band with the maximum absorbance at 655–661 nm (the wavelength depends on the solvent) and a nearby less visible band at 613–622 nm, which corresponds to the absorption of BChl *d* only [26, 47, 48], are present in the long-wavelength spectral region.

The brown GSB form differs from the green one in featuring more intense absorption in the blue-green region of visible wavelengths [49]. The short-wavelength band of the brown GSB form, which has BChl *e* as its primary photosynthetic pigment, consists of three overlapping bands with maxima at 412–415 and 430–434 nm (depending on the solvent), which correspond to the absorption of BChl *e* and carotenoids, and a well-pronounced band with its maximum at 466–479 nm (Fig. 1, *b*). The primary contributor to the latter band is a specific carotenoid (isorenieratene) [48]. The long-wavelength region of the spectrum of BChl *e*, just as the one of BChl *d*, features a well-pronounced absorption band with the maximum absorbance at 655–661 nm (the wavelength depends on the

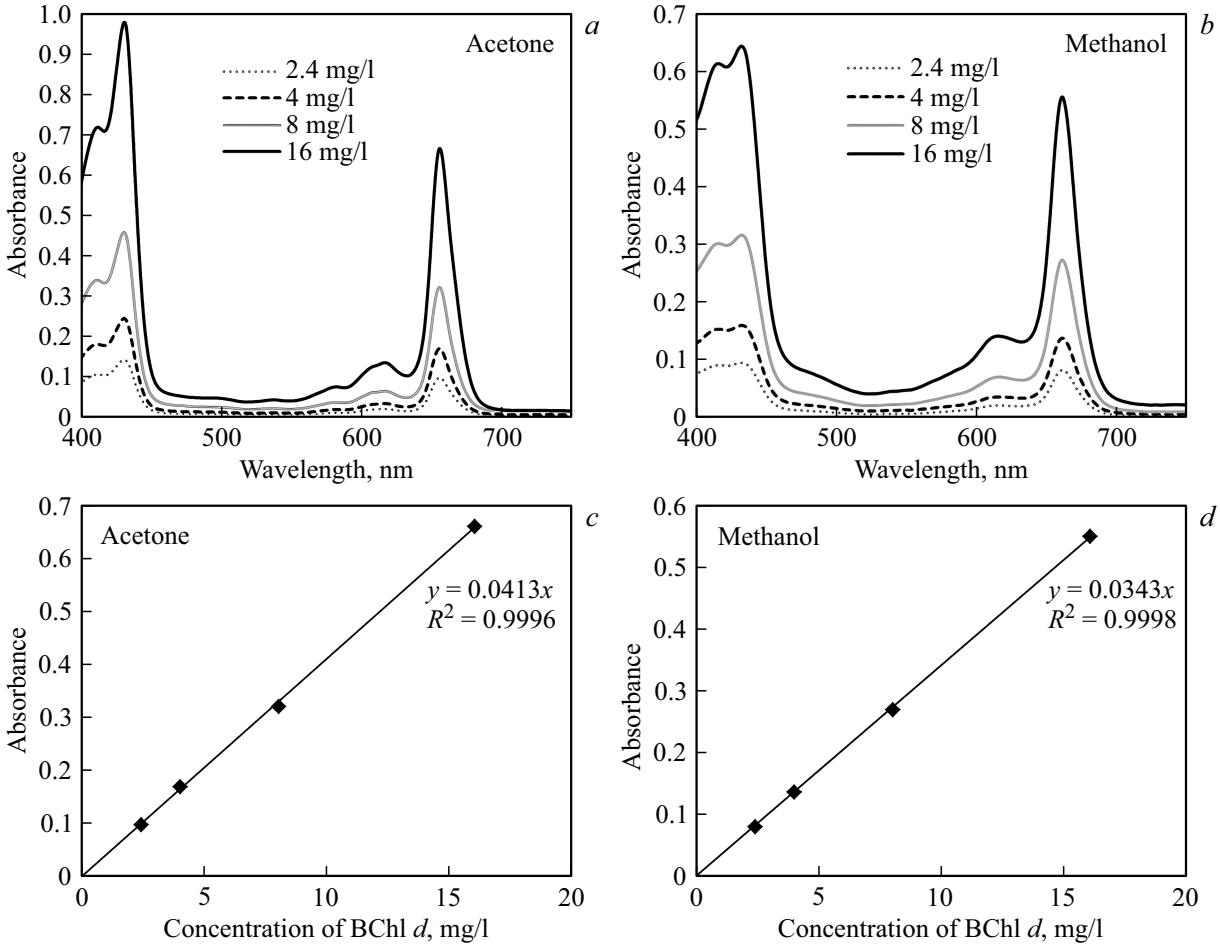

Figure 1. Absorption spectra of extracts of BChl *d* in acetone (*a*) and methanol (*b*) solutions. Dependences of absorbance at the maximum of the long-wavelength absorption band of BChl *d* on the concentration in acetone (*c*) and methanol (*d*).

solvent), which corresponds to the absorption of BChl *e* only [27,48], and a band with a lower absorbance with its maximum at 612–618 nm (Fig. 2).

2.2. Calculation of extinction coefficients of BChls *d* and *e*

The BChl concentrations in „base“ extracts were calculated by inserting the values of absorbance at the maxima of long-wavelength absorption bands of BChls *d* and *e* in acetone into formula (3). The absorbance at a wavelength of 655 nm (0.67) was used for BChl *d*, while an absorbance of 0.36 at 654 nm was used for BChl *e*. Acetone was chosen as a solvent due to the fact that the extinction coefficients of BChls *d* and *e* in acetone are known [26,27]. The obtained BChl *d* and *e* concentrations were 0.016 g/l (16 mg/l) and 0.014 g/l (14 mg/l), respectively. The concentrations of diluted 50%, 25%, and 15% BChl *d* solutions were taken equal to 8, 4, and 2.4 mg/l, while the concentrations of diluted 50%, 25%, and 15% BChl *e* solutions were 7, 3.5, and 2.1 mg/l.

Dependences of absorbance at the maximum of the long-wavelength absorption band, which corresponds to the absorption of BChl *d* or *e* in extracts, on the calculated BChl concentration were plotted for „base“ extracts and their three dilutions (Figs. 1 and 2). The obtained dependences turned out to be linear and allowed us to calculate the slope coefficients of straight lines approximating the experimental data (Table 2). The unknown extinction coefficients were determined with the use of the slope coefficient of BChl *d* or *e* in acetone, tabular extinction coefficients of BChl *d* or *e* in acetone, and calculated slope coefficients in other solvents (see Section 1.5). The obtained values are presented in Table 2.

3. Spectrophotometric formulae for quantitative measurement of BChl in different solvents

The formula for calculation of the BChl concentration in an arbitrary solvent (with only one BChl type present in the solution) is written as

$$C = AD/l, \tag{5}$$

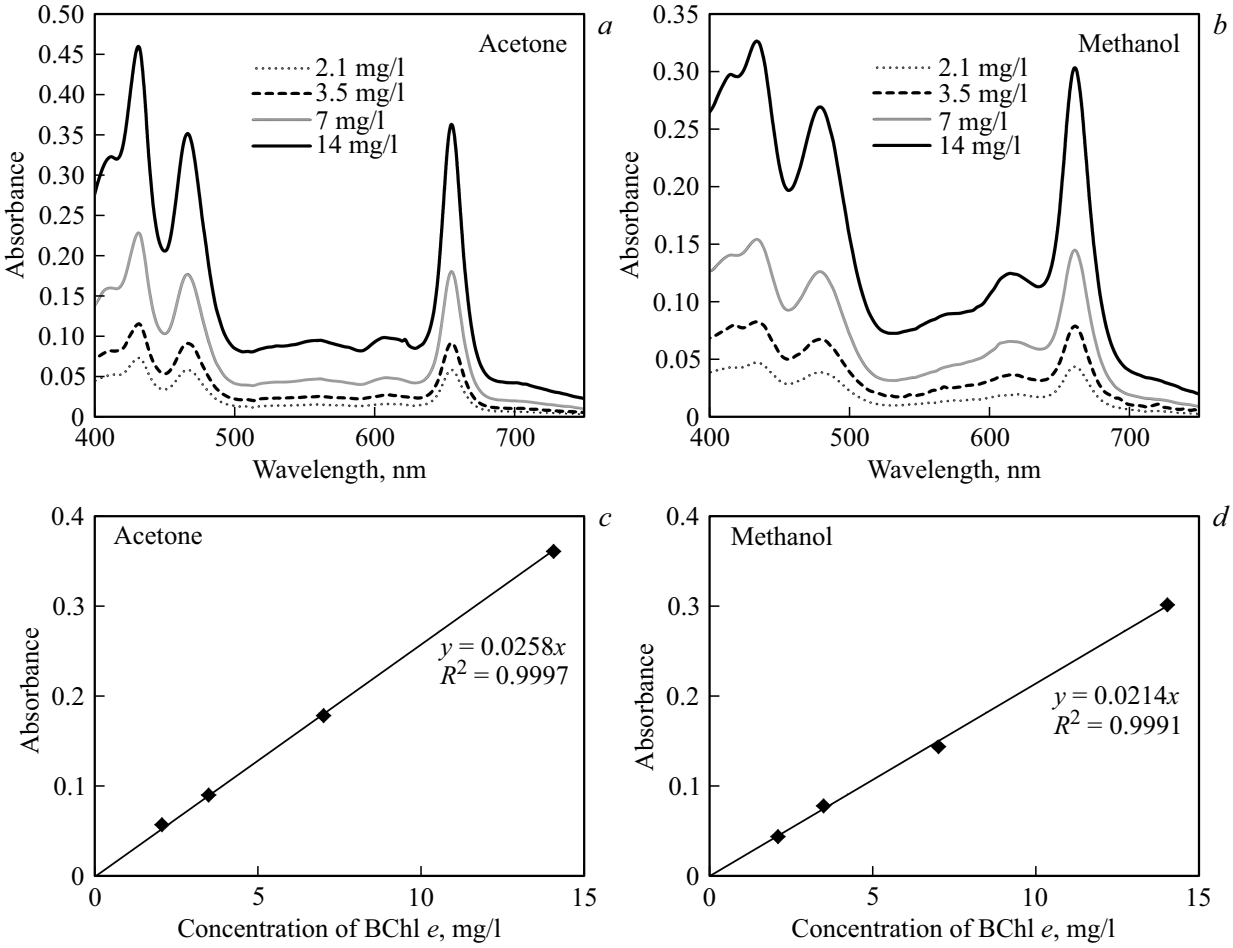

Figure 2. Absorption spectra of extracts of BChl *e* in acetone (*a*) and methanol (*b*) solutions. Dependences of absorbance at the maximum of the long-wavelength absorption band of BChl *e* on the concentration in acetone (*c*) and methanol (*d*).

where D is the absorbance at the absorption band maximum for a layer with thickness l (cm), and empirical coefficients of proportionality $A = \frac{1}{0.4343\varepsilon}$, which were calculated with the use of the corresponding extinction coefficient for a certain BChl and a specific solvent, are listed in Table 3. The BChl concentration determined this way is expressed in mg/l of solvent. The error of coefficient of proportionality A was calculated as $S_A = \frac{S_\varepsilon}{0.4343\varepsilon^2}$.

Discussion

The experimental dependences of absorbance on the pigment concentration turned out to be linear with the values of correlation coefficient R^2 varying from 0.98 to 1. This confirms that the Bouguer–Beer–Lambert law remains true for BChl solutions in the organic solvents used. No signs of aggregation of BChl *d* and *e* molecules were observed through to a concentration of 0.14 g/l. Thus, the relative method of calculation of extinction coefficients of BChls *d* and *e*, which was characterized in Section 1.5, provides correct data for the examined solutions.

The obtained BChl extinction coefficients were compared with literature data (see Table 2). The extinction coefficients at absorption maxima for BChl *d* in methanol and BChl *e* in ethanol are 81.5 ± 0.8 and 47.3 ± 0.9 l/g·cm, respectively, and agree almost perfectly with literature data (the deviation does not exceed 3.5%). The extinction coefficients of BChl *e* in a 7 : 2 acetone-methanol solution and in pure methanol were 11 and 13% higher, respectively, than literature values. This discrepancy may be attributed to the fact that the extinction coefficients used as references were determined at other wavelengths. The close fit between experimental data and literature values obtained with the use of chromatographic techniques [26,27] is indicative of validity of the obtained coefficients and applicability of long-wavelength absorption bands of extracts of bacterial cells in calculations of BChl concentration.

The extinction coefficients of chlorosome BChls *d* and *e* differ greatly, but are close to the coefficients of related compounds (Chls *a* and *b*). According to the obtained data, the extinction coefficient of BChl *d* at the maximum of the long-wavelength absorption band is 1.67, 1.69, and 1.72 times higher than the extinction coefficient of BChl *e*

Table 3. Coefficient A for spectrophotometric calculation of the concentration of BChls d and e in organic solvents

Solvent	Coefficient A for BChl d, (mg·cm)/l	Coefficient A for BChl e, (mg·cm)/l
Acetone	23.50 ± 0.22	39.3 ± 0.7
Acetone-ethanol (7 : 2)	27.9 ± 0.3	42.7 ± 0.9
Acetone-methanol (7 : 2)	29.8 ± 0.3	41.2 ± 0.8
Isopropanol	27.5 ± 0.3	41.0 ± 0.8
Ethanol	28.3 ± 0.3	48.7 ± 0.9
Methanol	28.2 ± 0.3	47.4 ± 0.9

in acetone, methanol, and ethanol, respectively. The ratio of extinction coefficients of Chls a and b is 1.72 in acetone and 1.88 in ethanol and methanol (Table 2). The similarity of spectral properties of photosynthetic pigments of bacterial and plant cells stems from the similarity of their chemical structures. Bacteriochlorophylls d and e, just as Chls a and b, belong to the group of phytochlorins and have one reduced pyrrole ring in the chemical structure. Bacteriochlorins such as, e.g., BChl a have two reduced pyrrole rings [45]. In a solution, the absorption spectra of BChl d are very similar to those of Chl a, and the absorption spectra of BChl e are very similar to Chl b spectra [18]. However, owing to certain structural differences (e.g., the presence of a methyl group, which may contribute to distortion of the porphyrin ring plane, in BChl e and the lack of a vinyl group, which is found in Chl b), the extinction coefficients of BChl e and Chl b differ [27]. According to the results of our calculations, the extinction coefficients of BChl e in acetone and methanol are 1.14 times greater than the tabular extinction coefficients of Chl b, while the extinction coefficients of BChl d in acetone and methanol are 1.11 and 1.02 times greater, respectively, than the tabular extinction coefficients of Chl a.

It should be stressed once more that the use of the extinction coefficient of BChl d for estimation of BChl of brown GSB, which contain BChl e, is incorrect. When working with extracts prepared from a water sample with a mixture of bacterial cells of both types, one needs first to separate the contributions of two BChls (e.g., following the procedure outlined in [31]) and then to calculate separately the concentrations of BChls d and e with the use of extinction coefficients determined for a specific solvent.

A 7 : 2 mixture of acetone and methanol was used successfully as a solvent for BChl extracts, and the extinction coefficient for BChl e in acetone-methanol (7 : 2) has been determined in [27]. However, methanol is toxic and difficult to procure, and a solvent with methanol replaced by the same amount of ethanol has also been examined. Since the extinction coefficients for two acetone mixtures differ little in magnitude, we propose to use a less toxic 7 : 2 mixture of acetone and ethanol for the preparation of extracts in BChl concentration measurements.

Conclusion

The extinction coefficients of BChl d in the long-wavelength absorption band (655–661 nm) in such solvents as ethanol, isopropanol, acetone-ethanol (7 : 2), and acetone-methanol (7 : 2) and the extinction coefficients of BChl e in isopropanol and acetone-ethanol (7 : 2) have been measured for the first time. The determination of light-absorbing properties of cellular pigments in these solvents opens up the opportunity to work with various solvents used in biological studies and chromatography and substitute the common acetone-methanol solution with a less toxic acetone-ethanol one. The obtained extinction coefficients at the long-wavelength absorption maxima of BChls allow one to calculate the BChl concentration in extracts, since carotenoids do not absorb light in this region. A general formula for spectrophotometric calculation of concentrations was presented, and the needed coefficients were determined.

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Conflict of interest

The authors declare that they have no conflict of interest.

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