

Detailed analytical procedures

Chlorophyll a, b, and carotenoids content measured

The measurements of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids (Car), more polar carotenoids (MPCar) and low polar carotenoids (LPCar) were carried out using an extraction-photometric method. The extraction of pigments from microalgae was carried out with 80.0% acetone. For this purpose, 5.0 mg of biomass separated from the medium by centrifugation (10 min; 3,000 rpm; 530× g) was subjected to 3-fold freezing-defrosting to destroy the cell envelopes. After that, 4.0 mL of acetone was added to create a ratio of biomaterial-extractant 1:80 (w:v) and additionally homogenised the biomass by rubbing with quartz sand. The resulting mixture was hermetically sealed and left for 24 hours at a temperature of 25.0 °C in a dark place for the complete extraction of pigments. Further, the acetone extract was separated from the sediment by centrifugation (10 min; 10,000 rpm; 6,000× g; 4.0 °C). The absorption intensity of the pigment extract was analysed on the spectrophotometer Ulab 102 (Ulab, China) at wavelengths 663,4 and 646.6 nm, which corresponds to the absorption maximum for Chl *a*, *b*. The chlorophyll content was expressed in mg g⁻¹ of dry biomass; calculations were carried out according to the following formulas:

$$\begin{aligned}\text{Chl } a &= 12.25 E_{663,6} - 2.55 E_{646,6} & (\mu\text{g mL}^{-1}) \\ \text{Chl } b &= 20.31 E_{646,6} - 4.91 E_{663,6} & (\mu\text{g mL}^{-1}) \\ \text{Chl } a+b &= 17.76 E_{646,6} + 7.34 E_{663,6} & (\mu\text{g mL}^{-1})\end{aligned}$$

Carotenoids were determined using extraction-photometric method. The method involves extracting the sum of the pigments from the biomass by 80.0% acetone, with further measurement of the maximum absorption of the carotenoid fraction at 440.5 nm and also takes into account the effect of absorption of chlorophyll *a*, *b*. Analysis of the extract was carried out on the spectrophotometer Ulab 102 (Ulab, China). Concentrations were expressed in mg g⁻¹ of dry biomass and calculated using the formula:

$$\text{Car} = 4.69 E_{440.5} - 0.267 \text{ Chl } a+b \quad (\mu\text{g mL}^{-1})$$

Pigment extract was obtained by adding 4.0 mL of 80.0% acetone to 5.0 mg of biomass separated from the medium by centrifugation (10 min; 3,000 rpm; 530× g). The biomass was previously subjected to 3-fold freezing-defrosting and 24-hour extraction at a temperature of 25.0 °C in a dark place. The sediment was then separated by centrifugation (10 min; 10,000 rpm; 6,000× g; 4.0 °C).

Next, we mixed the above acetone extract with 2.5 ml of hexane, stirred, and left in the dark until the phase separation. The upper phase contains less polar compounds dissolved in hexane, and the lower phase contains more polar compounds dissolved in acetone. The lower fraction was collected separately, and optical density measurements were performed at 440.5, which corresponds to the maximum absorption of more polar carotenoids (MPCars). We evaporated the upper hexane fraction in vacuum, and dissolved the dry residue with 80% acetone, destroyed chlorophyll molecules by adding 50 µl of 12.5% HCl solution. Next, we measured the absorption at 470, which are the main absorption maxima of less polar carotenoids (LPCars). To calculate the concentrations, we used the equations below.

$$\begin{aligned}\text{MPCar} &= 4.69 E_{440.5} - 0.267 \text{ Chl } a+b & (\mu\text{g mL}^{-1}) \\ \text{LPCar} &= (1000 E_{470} - 4.28 E_{665.4} - 4.78 E_{653.4})/164 & (\mu\text{g mL}^{-1})\end{aligned}$$

The content of pigments was expressed in mg g⁻¹ of dry biomass.

Lipids and protein content measurement

The determination of the total lipid content was carried out by the gravimetric method. A hexane-propane-2-ol mixture at a volume ratio of 3:2 was used for lipid extraction [25]. To achieve this, 20 mg of separated biomass was transferred to a pre-weighted sample bottle with a lid. Freeze-defrost and dry in a vacuum at 60 °C until a constant mass is obtained and weighed to determine the mass of the residue. The dry residue was extracted five times with a mixture of hexane-propane-2-ol: 2.0; 1.0; 1.0; 0.5; 0.5 mL. Before extraction, 1 mL of 0.9 % NaCl solution was added to the dry residue for better separation of the lipophilic and hydrophilic phases. To drain the extract, the top hexane layer was transferred by decantation to a 25 mL chemical glass, into which the 1 mm waterless Na₂SO₄ layer was previously poured. The extract was poured into a preweighted sample bottle, and a glass with precipitation was washed twice with hexane. The extracts were combined and evaporated under vacuum at 60 °C. The dry lipid fraction was weighed and expressed in mg g⁻¹ DW.

For protein content determination we used Olson's protocol [26], which is based on the bicinchoninic method. To obtain the protein extract of 10 mg of biomass separated by centrifugation from BBM medium, 3 times of freezing-defrosting and homogenization in 1 mL of ethanol was carried out to remove lipophilic substances. The resulting homogenate was centrifuged (10 min; 10,000 rpm; 6,000 g; 4 °C), after which the supernatant liquid was decanted and the residue was resuspended in 1 mL of a phosphate buffer (0.1 M; pH 7.5) containing 0.5 % sodium dodecyl sulfate by mass. The mixture was left in a hermetically sealed vial for 12 hours at 25 °C for complete protein extraction. The homogenate was centrifuged (10 min; 3,000 rpm; 530 g), and a supernatant was used to analyse protein content. The optical density of the solution was then measured at 562 nm on the ULab 102 spectrophotometer. The calibration curve was constructed according to the standard solution of bovine serum albumin (Thermo Scientific, the United States). The protein content was expressed in mg g⁻¹ DW.

Measurement of ascorbic acid, phenolic compounds and α -tocopherol content

The phenolic compound (PhenC) content of the samples was determined according to the Folin-Chocalteu colorimetric method according to a protocol described by Vazquez et al. [27]. The determination was based on an acidified (chlorophyll-destroying) trichloroacetic acid (TCA) methanol extract of phenolic compounds from microalgae. To achieve this, 10 mg of dry microalgae biomass were placed in a sealed vial with a lid and 0.5 mL of 50 % methanol (LenReaktiv, Russia) was added, containing 1.0 % TCA. The vial was blown with argon, sealed with a lid, and extracted at 80 °C for 20 min. The vials were cooled and centrifuged at 15 min at 4,000 rpm (600 g) to precipitate insoluble components. The 0.20 mL aliquot was then placed in a Falcon vial and 100.0 μ L of Folin-Chocalteu phenolic reagent (Scharlab, Barcelona, Spain), 1.0 mL of Na₂CO₃ solution (20.0 %, w/v) were added. The vials were placed in a dark place at room temperature for 30 min. The optical density of the solution was then measured at 760 nm on the ULab 102 spectrophotometer. The total phenolic compounds content was quantified with a calibration curve obtained using a standard gallic acid solution (Merck, Germany) in the concentration range of 1 to 20 mg L⁻¹. The concentration is expressed in galic acid equivalents mg at 1.0 g of DW (GAE mg/g⁻¹ DW).

The method of Kampfenkel et al. in the Zuffellato-Ribas et al. modification [28] was used to determine the content of ascorbic acid. For this purpose, 10 mg of microalgae biomass had used, which was previously been frozen and defrosting in a centrifugal plastic vial with a lid. Also, 1 mL of 10 % trichloroacetic acid (LenReaktiv, Russia) was added to the biomass and homogenised for 10 min at 4.0 °C. Furthermore, 800 μ L of 42 % H₃PO₄, 800 μ L of 4.0 % 2,2'-dipyridyl (Sigma-Aldrich, USA) (in 70 % ethanol) and 400 μ L of 3 % FeCl₃ (in H₂O) were introduced into the incubation mixture. The mixture was incubated at 42 °C for 45 min and then centrifuged (5 min; 5,000 rpm; 800 g, 4 °C). The optical density of the solution was then measured using a 525 nm. A calibration curve was used to quantify the optical density (mg g⁻¹ DW). The standard used was a calibration solution made of crystalline ascorbic acid (Supelco, Germany).

To prepare a sample, 30 mg DW of microalgae biomass was previously saponified at 80–85 °C in a KOH solution in ethanol with the addition of 20 mg of ascorbic acid (an antioxidant). α -Tocopherol was isolated from the resulting hydrolysate by sequential extraction with diethyl ether in volumes of 2 mL, 1 mL, and 1 mL. To remove the remaining KOH, the extract was washed sequentially with distilled water. For this purpose, after adding 2 mL of water to the extract, the mixture was shaken and left for 5 min to separate the lipophilic and hydrophilic fractions; the lower aqueous layer containing potassium hydroxide

was then removed with a syringe and needle. The washing procedure was repeated until the water layer showed a neutral reaction on universal indicator paper. Next, the extract was dried by freezing out the water. The washed, combined extract was placed in a freezer at -18 °C for 1 h, causing water to precipitate as ice crystals. After that, the combined extract was taken into a syringe without water crystals, and filtered through a membrane filter with a pore size of 0.22 µm. The resulting extract was evaporated in a vacuum at 45–55 °C. A RE-201C rotary evaporator (Titat Tech, China) was used in conjunction with a 2VP-1 plate-rotor vacuum pump (Stegler, China) to create a negative pressure (0.067 Pa) in the vacuum installation. The heating was carried out in a water bath complete with a rotary evaporator. The dry residue was dissolved in 1 mL of methanol for analysis. The volume of the injected sample was 10 µL.

The content of α -tocopherol in biomass was determined by reverse-phase HPLC using a Chromatron-1411 liquid chromatograph (JSC Labtech, Russia) with a UV spectrophotometric detector. We described the definition algorithm in detail in our previous work [Maltseva et al., 2026]. Reverse-phase HPLC determined the content of α -tocopherol in biomass. Determination of α -tocopherol was carried out on a Chromatron-1411 liquid chromatograph (JSC Labtech, Russia) with a spectrophotometric UV detector. α -Tocopherol detection was performed at a wavelength of 292 nm. An Inspire C18 column (5 µm 150 × 4.6 mm) was used as a carrier of the stationary phase. A mixture of methanol:water was used as the mobile phase in the ratio 96:4 (v:v), containing 2.5 mM acetic acid/sodium acetate [29]; the mixture was supplied at a rate of 1 mL min⁻¹. The separation time was 10 min. During separation, the column temperature was 30 °C. The retention time of α -tocopherol was determined by the retention time of a standard sample from a Merck 613,424 kit (Millipore, the USA). For the quantitative determination of α -tocopherol, the calibration curve method was used, which was constructed based on the height and area of peaks obtained by chromatography of calibration solutions with a final concentration of 10–100 µg mL⁻¹. Calibration solutions were prepared by diluting a standard solution obtained by diluting α -tocopherol from a Merck kit (Millipore, the USA) in methanol, with an initial concentration of 1 mg mL⁻¹. The concentration was expressed in µg per g DW.