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ORIGINAL ARTICLE

Rapid screening test to estimate temperature optima for microalgae growth using photosynthesis activity measurements

Karolína Ranglová[1,2] · Gergely Ernö Lakatos[1] · João Artur Câmara Manoel[1,3] · Tomáš Grivalský[1] · Jiří Masojídek[1,3]

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Abstract

We have worked out a rapid 1-day test based on photosynthesis measurements to estimate suitable growth temperature of microalgae cultures. To verify the proposed procedure, several microalgae—*Chlorella*, *Nostoc*, *Synechocystis*, *Scenedesmus*, and *Cylindrospermum*—were cultured under controlled laboratory conditions (irradiance, temperature, mixing, CO_2, and nutrient supply) to find the optima of photosynthetic activity using the range between 15 and 35 °C. These activities were recorded at each temperature step after 2 h of acclimation which should be sufficient as oxygen production and the PQ cycle are regulated by fast processes. Photosynthetic activity was measured using three techniques—oxygen production/respiration, saturating pulse analysis of fluorescence quenching, and fast fluorescence induction kinetics—to estimate the temperature optima which should correspond to high growth rate. We measured all variables that might have been directly related to growth—photosynthetic oxygen evolution, maximum photochemical yield of PSII, F_v/F_m, relative electron transport rate $rETR_{max}$, and the transients V_j and V_i determined by fast fluorescence induction curves. When the temperature optima for photosynthetic activity were verified in growth tests, we found good correlation. For most of tested microalgae strains, temperature around 30 °C was found to be the most suitable at this setting. We concluded that the developed test can be used as a rapid 1-day pre-screening to estimate a suitable growth temperature of microalgae strains before they are cultured in a pilot scale.

Keywords Chlorophyll fluorescence · Electron transport rate · Microalgae · Photosynthesis measurements · Rapid test · Temperature optimisation

Abbreviations

Chl	Chlorophyll
DCMU	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
DM	Dry mass
F_0, F_v, F_m	Minimal, variable, and maximal fluorescence in dark-adapted state
F_v/F_m	Maximal photochemical yield of PSII
POE/R	Photosynthetic oxygen evolution and respiration
OJIP curve	Fast Chl fluorescence induction kinetics
PAM	Pulse-amplitude modulation
PAR	Photosynthetically active radiation
PQ	Plastoquinone pool
PSII	Photosystem II
PTFE	Polytetrafluoroethylene
rETR	Relative electron transport rate through PSII
RLC	Rapid light-response curve

Dedicated to the memory of Prof. Ivan Šetlík

✉ Karolína Ranglová
ranglova@alga.cz

[1] Centre Algatech, Laboratory of Algal Biotechnology, Institute of Microbiology of the CAS, Třeboň, Czech Republic

[2] Faculty of Agriculture, University of South Bohemia, České Budějovice, Czech Republic

[3] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Introduction

Microalgae are fast-growing photosynthetic organisms since their cell doubling time can be as little as few hours. Growth of microalgae is affected by various environmental variables such as light, temperature, nutrition, pH, presence of contaminants and others (Cho et al. 2007; Bernard and Rémond 2012). Light intensity and temperature are the most important

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environmental variables that influence microalgae growth. As concerns temperature regime, some microalgae strains tolerate a broad range of temperatures (15 to 40 °C; e.g. *Chlorella*), while marine diatoms such as *Phaeodactylum* usually require stricter regulation (20 to 25 °C), where at 30 °C growth is completely inhibited. For the majority of freshwater microalgae, the optimum temperature ranges from 25 to 35 °C (Masojídek and Torzillo 2014). Each microalgae strain is characterized by an optimal growth temperature which should correlate to its photosynthetic activity indicating its maximum photosynthetic efficiency (Béchet et al. 2013; Ras and Steyer 2013; He et al. 2018). Sub-optimal (or supra-optimal) temperatures usually cause a decrease in photosynthetic activity and subsequently also microalgae growth (Torzillo et al. 1991; Benavides et al. 2017).

Suitable cultivation regimes have to be worked out for each microalgae strain and particular cultivation unit because the optimum temperature may vary depending on the construction details (light path, mixing, etc.). Since the nineteenth century (e.g. Martinus Beijerinck in 1890) classical growth tests have been employed for optimisation of cultivation regimes determining changes of microalgae biomass density in illuminated cultivation vessels. Regular growth tests may take several days, or even weeks in order to combine various conditions. Later, in the 1980s, cross-gradient cultivation techniques were developed which significantly improved the estimation of the light/temperature regime. In these tests, microalgae cultures are usually grown in Petri dishes placed on a surface with a temperature and light intensity gradient. This approach can give a number of combinations of temperature and light regimes to be tested at the same time (Lukavský 1982). Still, it is rather a preliminary test which can take days and has the great disadvantage that the strains are grown on agar plates, not in mixed liquid media with a supply of CO_2.

During the growth of microalgae, monitoring of photosynthetic activity changes is advantageous to monitor the culture physiological status (Havlik et al. 2013; Malapascua et al. 2014). Recently, chlorophyll (Chl) fluorescence techniques (quenching and induction curve analyses) have been used in the rapid monitoring of photosynthetic activity to detect various unfavourable effects of environmental stressors on microalgae and to optimise culture growth (Babaei et al. 2017; Maxwell and Johnson 2000; Strasser et al. 2004; Baker 2008; Masojídek et al. 2011a, 2011b; Malapascua et al. 2014). Relative electron transport curves (rETR) measured by Chl fluorescence techniques were used to monitor the dependency of photosynthesis on culturing conditions, mostly irradiance and temperature (e.g. Torzillo et al. 1996, 1998; Kromkamp et al. 1998; Ralph and Gademann 2005; Enríquez and Borowitzka 2010; Malapascua et al. 2014; Benavides et al. 2017). Generally, the trends of rETR correlated well with the culture growth. Another monitoring approach is to examine fast fluorescence induction kinetics characterized by the fast chlorophyll fluorescence induction curve (so-called OJIP curve). While fluorescence quenching analysis gives information on the balance between photosynthetic electron transport and the Calvin-Benson cycle, fast fluorescence induction kinetics provides information on the reduction of the photosynthetic electron transport chain (Malapascua et al. 2014). Measurement of photosynthetic oxygen evolution (POE) provides similar data to that of Chl fluorescence techniques with one important advantage, the possibility to also monitor dark respiration (R) and correlate these to electron transport measurements by Chl fluorescence (Figueroa et al. 2003).

In this work, a fast preliminary test based on photosynthetic measurements (Chl fluorescence and POE) has been correlated with classical growth tests. The experiments showed which photosynthetic variables can be closely related to culture growth. Based on this comparison, the suitable growth regime (temperature) for microalgae strains could be estimated within 1 day.

Materials and methods

Strains

Several microalgae strains were used in laboratory trials. Four green microalgae (*Scenedesmus* MACC-677, *Chlorella* MACC-1 (Algal Culture Collection, Szechényi István University, Mosonmagyárovár, Hungary), *Scenedesmus almeriensis* CCAP 276/24 (University of Alméria, Spain), and *Chlorella vulgaris* R-117 (CCALA 1107, Culture Collection of Autotrophic Organisms, Institute of Botany, Třeboň, Czech Republic)) and four cyanobacteria strains *Cylindrospermum alatosporum* (CCALA 988 Culture Collection of Autotrophic Organisms, Institute of Botany, Třeboň), *Synechocystis* sp. PCC 6803 (Pasteur Collection, Paris), *Nostoc* MACC-612, and *Nostoc* MACC-683 (Mosonmagyaróvár Algal Culture Collection, Szechényi István University, Hungary) were used in laboratory experiments. The cultures were grown in BG-11 medium (Rippka et al. 1979). Further in the text, the strains are abbreviated as *Scenedesmus* 677, *Chlorella* 1, *S. almeriensis*, *Chlorella* R-117, *Cylindrospermum* 988, *Synechocystis* 6803, *Nostoc* 612 and *Nostoc* 683.

Cultivation equipment and conditions

Stock cultures were grown in glass tubes (400 mL) bubbled with air + 1% CO_2 in inorganic BG-11 medium under controlled laboratory conditions: biomass density was between 1 and 2 g/L, temperature was set to 25 °C and light intensity was 80–100 μmol/m^2 photons per s. Established cultures of each strain were diluted with fresh medium to the initial biomass

density of ~ 0.5 g/L DM and transferred into 100-mL glass test tubes (∅30 × 200 mm) which were submerged into a temperature-controlled water bath and exposed to the light intensity of 100 μmol/m^2 photons per s (Fig. 1).

Several cultivation units were used in parallel. The cultures were acclimated to temperatures in the range between 15 and 35 °C using 5° steps under continuous irradiance of 100 μmol/m^2 photons per s for 2 h. At each temperature step, a new batch from the stock culture was prepared. Photosynthetically active radiation (PAR) provided by a panel of high-frequency cool fluorescent tubes with adjustable light intensity (36 W/830 Lumilux, Osram, Germany) was measured at the front surface of the water bath. The photosynthetic performance of all strains at different temperatures was followed.

Then, the suitable cultivation regime was confirmed by a 5-day growth test carried out in triplicate in the temperature range (25–35 °C) close to the optimum under continuous moderate irradiance (100 μmol/m^2 photons per s).

Photosynthesis measurements

Photosynthetic activity of tested cultures was measured using three techniques—saturation pulse analysis of fluorescence quenching to record rapid light-response curves (RLC), fast fluorescence induction kinetics (OJIP), and photosynthetic oxygen evolution and respiration (POE/R) to construct steady-state light-response curves. All records were analysed to determine photosynthesis variables.

Microalgae samples taken from the glass tubes were diluted to 0.2 to 0.3 g/L DM (corresponding to 5 to 7 mg/L Chl) with growth medium, dark adapted for 10 min, and measured *off-situ*. In this way, light re-absorption problems in dense cultures were prevented by dilution and sufficient illumination was available to reduce the plastoquinone pool (closure of PSII reaction centres). Photosynthesis measurements were carried out using standardised procedures at temperatures corresponding to growth values. Data were recorded in triplicate by measuring of three separate samples ($n = 3$).

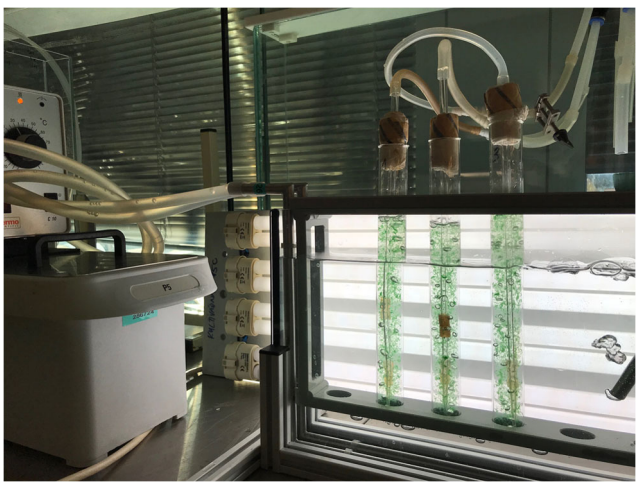

Fig. 1 Laboratory cultivation unit with side illumination panel fitted with high-frequency fluorescent tubes. Glass 100-mL columns with microalgae cultures are submerged in temperature-controlled water bath

Rapid light-response curves

Rapid light-response curves (RLCs) of microalgae samples taken from the cultures were measured in a light-protected measuring chamber with mixing (3-mL glass cuvette with light path of 10 mm) using a pulse-amplitude modulation fluorimeter (PAM-2500, H. Walz, Germany). A series of stepwise increasing irradiance intensities (red LEDs; 0–2700 μmol/m^2 photons per s) were applied in 20-s intervals to obtain the steady-state fluorescence level F' and then a saturating pulse (> 10,000 μmol/m^2 photons per s, 0.6-s duration) was triggered to reach the maximum F_m'. At each step, the actual PSII photochemical quantum yield in the light, Y_{II} was determined as $(F_m' - F')$ F_m' where F_m' is the maximum fluorescence level and F' is the steady-state fluorescence in the light-adapted state at respective irradiance level. Analysis of RLCs was used to estimate changes of the relative electron transport rate through PSII, rETR, which was calculated by multiplication of the actual photochemical efficiency Y_{II} and the photosynthetically active radiation E_{PAR}, $rETR = Y_{II} \times E_{PAR}$ (dimensionless) (e.g. Hofstraat et al. 1994; Ralph and Gademann 2005; White et al. 2011). In order to determine $rETR_{max}$ and the irradiance saturating photosynthesis, light response curves were fitted to the non-linear least-squared regression model by Eilers and Peeters (1998).

The minimum and maximum fluorescence levels (F_0, F_m) were determined using a weak modulated light (< 0.15 μmol/m^2 photons per s, frequency of 0.5–1 kHz) in the dark-adapted samples (actinic irradiance = 0, first step of RLC). The maximal PSII quantum yield was calculated as the ratio of variable and maximal fluorescence, $F_v/F_m = (F_m - F_o)$ F_m; it indicates the capacity of the system to absorb light through the reaction centres and the light-harvesting complex and expresses the maximum quantum efficiency of primary photochemistry (Strasser et al. 2004). For cyanobacteria, the plastoquinone pool (PQ) is shared by photosynthetic and respiratory electron transport chains. Thus, the "true" F_m has to be determined under low actinic illumination (~ 150 μmol/m^2 photons per s) in the presence of 10^{-5} M herbicide DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) which blocks electron transport behind the PSII complex. In the absence of DCMU, the apparent F_m in dark-adapted culture samples are usually 15–20% lower than the "true" value.

Fast chlorophyll fluorescence induction kinetics

Fast chlorophyll fluorescence induction kinetics (OJIP curves) were measured *off-situ* using a portable fluorimeter (AquaPen

AP-100, P.S.I. Ltd. Brno, Czech Republic). The sample for Chl fluorescence induction kinetics (OJIP curve) was followed in diluted (0.2 to 0.3 g/L DM) sample, dark-adapted for 10 min before measuring. The samples were transferred to a 3-mL measuring cuvette (light path of 10 mm) which was mounted in a light-protected holder in front of the detector (adjustable measuring light pulses, ~2.5 μs) while red LEDs served as high-intensity actinic light from both sides of the cuvette (up to 3000 μmol/m^2 photons per s), perpendicular to the detector. The OJIP curve was measured in the time range between 50 μs and 1 s when the signal rises rapidly from the origin (*O*) to highest peak (*P*) via two inflections—*J* and *I* (Strasser et al. 1995). The *O* point (50 μs) of the fluorescence induction curve represents a minimum value (designated as constant fluorescence yield F_0) when PQ electron acceptors (Q_A and Q_B) of the PSII complex are oxidized. The inflection *J* occurs after ~2–3 ms of illumination and reflects the dynamic equilibrium (quasi-steady state) between Q_A and Q_A^-. The *J*–*I* phase (at 30–50 ms) corresponds to the closure of the remaining centres, and the *I*–*P* (ending at about 300–500 ms) corresponds to full reduction of the plastoquinone pool (equivalent to maximum fluorescence level F_m) (Strasser et al. 2004; Goltsev et al. 2016). From the fluorescence levels at the *J* and *I* points, the variables V_j and V_i were calculated as follows:

$$V_j = (F_{2ms} - F_0)/(F_m - F_0) \text{ and } V_i = (F_{30ms} - F_0)/(F_m - F_0)$$

Measurement of oxygen production and respiration

Photosynthetic oxygen evolution (POE) was measured polarographically using a temperature-controlled chamber with adjustable illumination and mixing connected to temperature-controlled bath and a control unit (Oxygen Monitoring System Oxylab+; Hansatech, UK). Samples taken from experimental cultures were adapted for 10 min in the dark at the desired temperature. Light-response curves were recorded using a stepwise increasing light intensity of 0, 200, 400, 600, 1200, and 1800 μmol/m^2 photons per s where each step was adjusted to 2 min. The first step (2 min in the dark) provided the value of the dark respiration. Oxygen production and respiration were calculated in micromole of O_2 per milligramme (Chl) per hour.

Analytical procedures

Biomass was measured as dry mass (DM) by filtering 5 mL of culture samples on pre-weighed glass microfiber filters (GC-50). The pre-weighted filters with the cells were washed twice with deionized water, dried in an oven at 105 °C for 8 h, then transferred to a desiccator to equilibrate to laboratory temperature and weighed (precision of ± 0.01 mg). The growth rate $\mu = (\ln X_2 - \ln X_1) \quad \Delta t$ (1/d) of microalgae cultures was calculated over the whole period of 5-day growth trial to confirm the optimal temperature obtained from measuring changes in the photosynthetic apparatus.

Chlorophyll concentration was determined spectrophotometrically in methanol extracts. Samples of 500 μL were collected in 2-mL Eppendorf tubes and centrifuged at 13,000 rpm for 3 min (centrifuge Minispin, Eppendorf). The pellet was resuspended in 500 μL of 100% methanol, 100 μL of sea sand was added, and the tubes were put into a laboratory ultrasound bath (Kraintek 6) for 2 min, then cooled down in an ice bath and centrifuged at 10,000 rpm for 1 min. If necessary, the extraction was repeated several times until the pellet was colourless. The absorbance of the combined supernatants from all extraction steps was measured at 665 and 750 nm using a high-resolution spectrophotometer (UV 2600 UV-VIS, Shimadzu, Japan, slit width of 0.5 nm) and the concentration of chlorophyll was calculated according to Wellburn (1994).

Irradiance measurements

Photosynthetically active radiation (PAR) was measured as 10 s averaged values using a LI-250A light meter (Li-Cor, USA) with a flat quantum sensor (LI-190SA, cosine-corrected up to 80° angle of incidence).

Statistical analysis

Sigma Plot 11.0 was used to determine significant differences between treatments. One-way ANOVA and the Holm-Sidak test were conducted for every binary combination of data. *P* values lower than 0.05 were considered significantly different.

Results

The laboratory experiments were carried out in order to find suitable culturing conditions (temperature) of several microalgae strains using fluorescence measuring techniques. At each temperature step, a new batch from the stock culture was taken and acclimated for 2 h to the irradiance of 100 μmol/m^2 photons per s. Then, photosynthetic activity was measured as POE/R, RLC, and OJIP kinetics. In the following series of trials, growth tests were carried out where microalgae were cultured for 5 days at different temperatures (25, 30, and 35 °C).

The maximum photochemical yield of PSII, F_v/F_m (measured in the presence of DCMU) was recorded in the range from 15 to 35 °C in cultures of cyanobacteria strains *Nostoc* 612, *Nostoc* 683, *Cylindrospermum* 988, and *Synechocystis* 6803. The F_v/F_m values ranged from 0.17 at low temperatures

up to 0.63 at favourable temperature (Fig. 2a). The highest value (0.63) was observed in the *Nostoc* 612 culture while all the other strains showed lower maxima between 0.31 and 0.38. In all cyanobacteria strains, the maximum values of F_v/F_m were measured when the temperature was between 26.7 and 28.3 °C. It is important to note that the F_v/F_m values increased from low up to optimum temperature and then decreased as the condition became unfavourable.

In the case of eukaryotic microalgae *Scenedesmus* 677, *Chlorella* 1, *S. almeriensis*, and *Chlorella* R-117, the course of F_v/F_m was different. Only *Scenedesmus* 677 showed increasing fluorescence values from low temperature up to the maxima at temperature 25 °C and then started to decrease (Fig. 2b). In all other cultures, little variation (near-flat curves) was found in the temperature dependence curves of F_v/F_m suggesting a broad temperature range for activity. Similar results were found in *S. almeriensis* where the curves of temperature dependence for F_v/F_m of about 0.7 were found between 10 and 40 °C (Sánchez et al. 2008). Thus, measurements of other photosynthetic variables were necessary to clarify the question of temperature dependence.

Light-response curves of relative electron transport rate (rETR) were recorded using saturating pulse analysis of fluorescence quenching to evaluate photosynthetic activity of microalgae cultures. The rETR–temperature dependence curve maximum values were calculated for all microalgae strains. Among cyanobacteria, the highest $rETR_{max}$ value of about 140 was found in *Nostoc* 612 at 30 °C, the other cyanobacteria strains showed much lower activities (Fig. 3a). These results reflected the F_v/F_m values measured for cyanobacteria strains (Fig. 2a). For eukaryotic microalgae, the highest $rETR_{max}$, 1350, was found in *S. almeriensis* at 25 °C, which was still about one order of magnitude higher than that in *Nostoc* 612 (Fig. 3b). The $rETR_{max}$ value

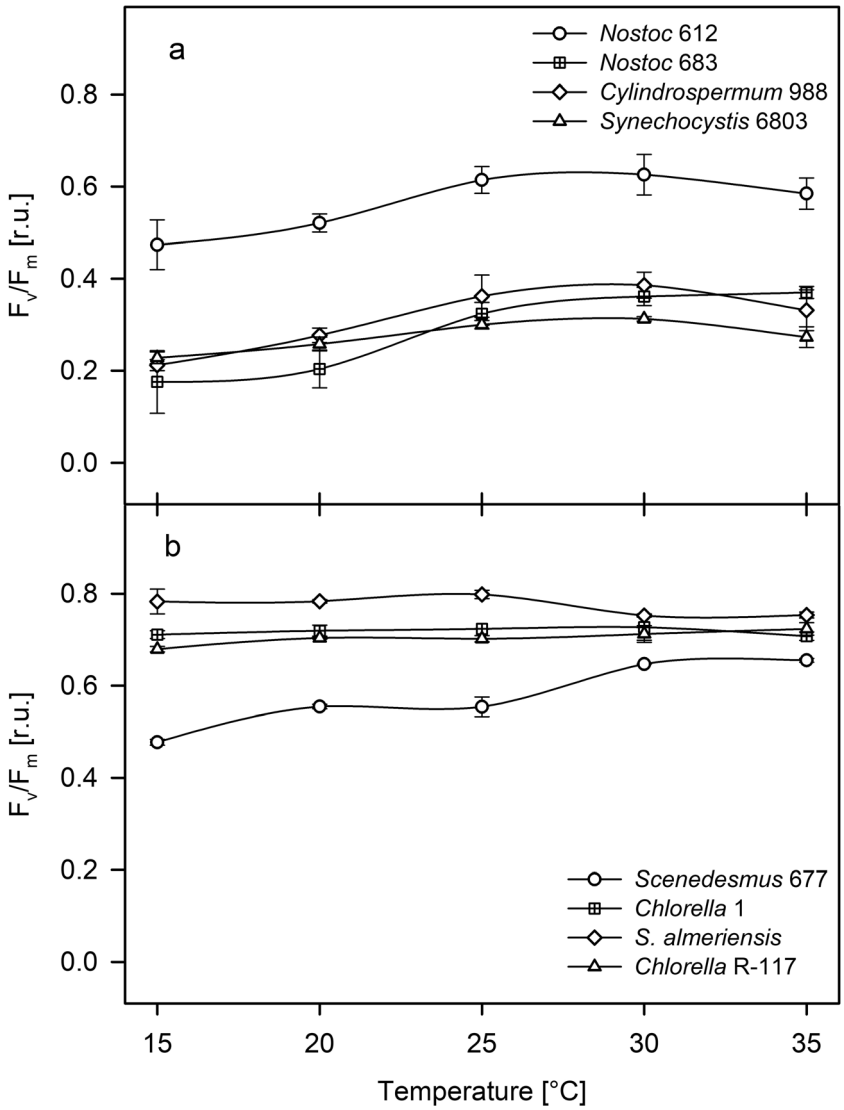

Fig. 2 Changes of the maximum photochemical quantum yield of PSII (F_v/F_m) as a function of temperature in the range of 15–35 °C for selected microalgae cultures. (a) *Nostoc* 612, *Nostoc* 683, *Cylindrospermum* 988, and *Synechocystis* 6803. (b) *Scenedesmus* 677, *Chlorella* 1, *S. almeriensis*, and *Chlorella* R-117. Values are presented as a mean ($n = 3$) with SD indicated by error bars

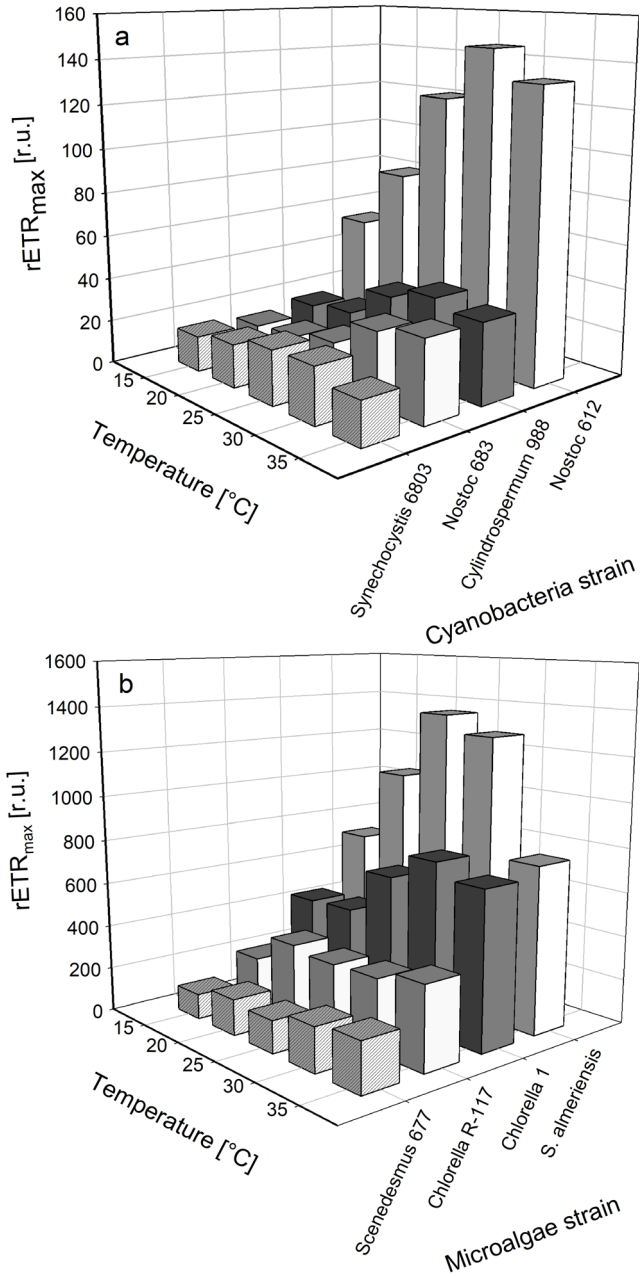

Fig. 3 Changes of the maximum electron transport rate $rETR_{max}$ (calculated from RLCs) as a function of temperature measured at 15, 20, 25, 30, and 35 °C for selected microalgae cultures. (a) *Nostoc* 612, *Nostoc* 683, *Cylindrospermum* 988, and *Synechocystis* 6803. (b) *Scenedesmus* 677, *Chlorella* 1, *S. almeriensis*, and *Chlorella* R-117

measured in the *Chlorella* 1 culture was much lower, about 770 (at 30 °C). In *Chlorella* R-117 and *Scenedesmus* 677 cultures, the $rETR_{max}$ values were lower, 375 and 224, respectively.

Fast fluorescence induction kinetics, the so-called OJIP curves indicating electron transport steps through the PSII complex, show the two inflection points *J* and *I*. These are represented by the V_j and V_i variables which indicate the redox status of the acceptor side of the PSII complex (downregulation of photosynthetic electron transport). If increased, V_j and V_i indicated a slowdown of electron transport due to overreduction of the plastoquinone pool. When the V_j and V_i variables were evaluated for all strains, the minimum values indicated the most favourable conditions (Fig. 4). The V_j and V_i variables were higher for cyanobacteria (0.6–0.9) as compared with V_j (0.3–0.4) and V_i (0.5–0.7) measured in eukaryotic microalgae. For *Nostoc* 612 and *Synechocystis* 6803, the lowest values indicating the favourable conditions were found in the range between 25 and 30 °C (Fig. 4a, d) while for *Cylindrospermum* 988 and *Nostoc* 683, the favourable conditions were achieved at 30 and 35 °C (Fig. 4b, c). The lowest value of both variables was measured when temperatures of 30 and 35 °C was set (Fig. 4e–h). The maxima of photosynthetic oxygen evolution (POE) and respiration were achieved under the conditions which can be considered suitable for photosynthetic oxygen production activity of individual microalgae strains. The highest activity was achieved in the range between 28.7 and 33.3 °C (Table 1).

In the second series of experiments, the microalgae cultures were grown in laboratory experiments at 25, 30, and 35 °C for 5 days to verify the temperature optima estimated from photosynthetic activity measurements (Fig. 5). The optimum temperature was estimated in the range between 30 and 35 °C for most of tested strains (*Nostoc* 612, *Nostoc* 683, *Cylindrospermum* 988, *Synechocystis* 6803, *Scenedesmus* 677, and *Chlorella* 1). Only for *S. almeriensis* and *Chlorella* R-117 the optimum growth temperature was 25 °C.

The growth rates μ of all microalgae strains were calculated over the whole period. The highest growth rate, 0.48 1/d, was found for *Nostoc* 612, followed by *Cylindrospermum* 988 at 0.33 1/d, *Chlorella* 1 at 0.28 1/d, *Scenedesmus* 677 at 0.27 1/d, and *Synechocystis* 6803 at 0.23 1/d when they were grown at 30 °C. Two strains—*S. almeriensis* and *Chlorella* R-117—showed the highest growth rates (0.25 and 0.29 1/d, respectively) at 25 °C. For *Nostoc* 683, the highest rate (0.28 1/d) was found at 35 °C.

Finally, the data from all measurements—maximum photochemical yield of PSII, F_v/F_m, $rETR_{max}$, fluorescence induction transients V_j/V_i, and POE/R—were summarised for each microalgae strain and averaged (Table 1). The photosynthesis optima for the examined strains were found in the range between 27.5 and 33.3 °C. We have not found any remarkable discrepancies between photosynthesis and growth optima, i.e. the averaged optima obtained from measurements of photosynthetic activity correlated with the regular growth tests for most of the strains. Only one exception was *Chlorella* R-117 where the mean temperature obtained from the measurement of photosynthetic activity was found to be 33.3 °C and the growth optimum obtained from regular cultivation test was found to be 25 °C. It is important to mention that *Chlorella* R-117 is used as a fast-growing

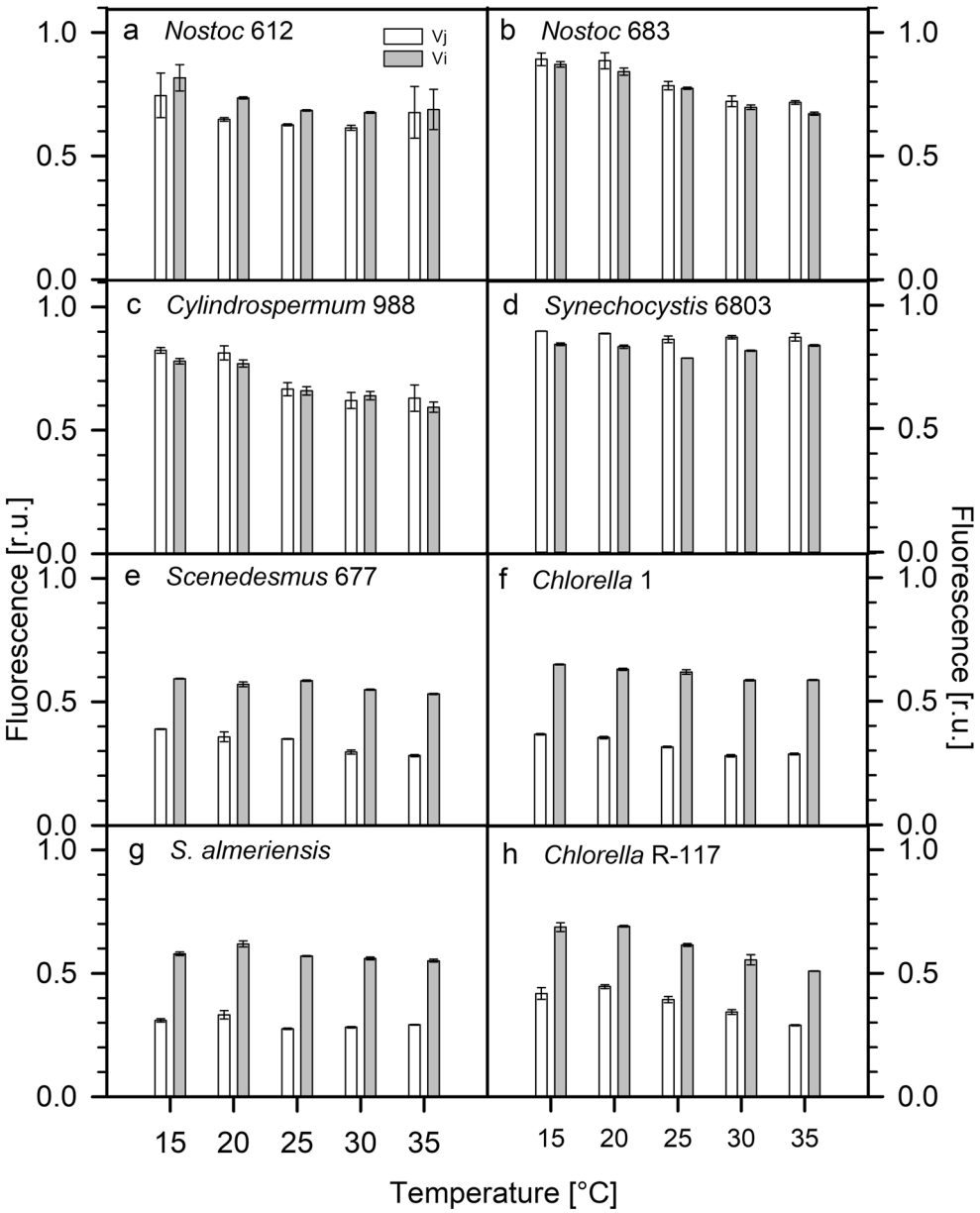

Fig. 4 Changes of the V_j and V_i variables (calculated from the curves of fast fluorescence induction kinetics) as a function of temperature at 15, 20, 25, 30, and 35 °C for selected microalgae cultures. (a) *Nostoc* 612, (b) *Nostoc* 683, (c) *Cylindrospermum* 988, (d) *Synechocystis* 6803, (e) *Scenedesmus* 677, (f) *Chlorella* 1, (g) *S. almeriensis*, and (h) *Chlorella* R-117. Values are presented as a mean ($n = 3$) with SD indicated by error bars

Table 1 Temperature optima for individual microalgae strains were estimated from measurements of the maximum photochemical yield of PSII, F_v/F_m, relative electron transport rate $rETR_{max}$, fluorescence induction transients V_j/V_i, and photosynthetic oxygen evolution and respiration POE/R. The temperature optima data for all variables—F_v/F_m, $rETR_{max}$, V_j/V_i, and POE/R—were then averaged (mean). The growth optima were verified in a 5-day cultivation trial. Values are presented as a mean ($n = 3$) with SD. The same letters mean that the values do not differ significantly from each other

Variable Temperature optima	F_v/F_m [°C]	$rETR_{max}$ [°C]	V_j, V_i [°C]	POE/R [°C]	Mean [°C]	Growth optimum [°C]
Nostoc 612	28.3 ± 2.9	30.0 ± 0	28.3 ± 2.9	28.3 ± 2.9	28.7 ± 2.2^{a,b}	30
Nostoc 683	28.3 ± 2.9	33.3 ± 2.9	33.3 ± 2.9	35.0 ± 0	32.5 ± 2.2^{a,b}	35
Cylindrospermum 988	28.3 ± 2.9	28.3 ± 2.9	33.3 ± 2.9	33.3 ± 2.9	30.8 ± 2.9^{a,b}	30
Synechocystis 6803	26.7 ± 2.9	28.3 ± 2.9	26.7 ± 2.9	31.7 ± 2.9	28.4 ± 2.9^{a,b}	30
Scenedesmus 677	33.3 ± 2.9	33.3 ± 2.9	33.3 ± 2.9	33.3 ± 2.9	33.3 ± 2.9^{a,b}	30
Chlorella 1	33.3 ± 2.9	35.0 ± 0	33.3 ± 2.9	31.7 ± 2.9	33.3 ± 2.2^{a,b}	30
S. almeriensis	25.0 ± 0	26.7 ± 2.9	26.7 ± 2.9	31.7 ± 2.9	27.5 ± 2.2^{a,b}	25
Chlorella R-117	31.7 ± 2.9	35.0 ± 0	33.3 ± 2.9	33.3 ± 2.9	33.3 ± 2.2^{a,b}	25

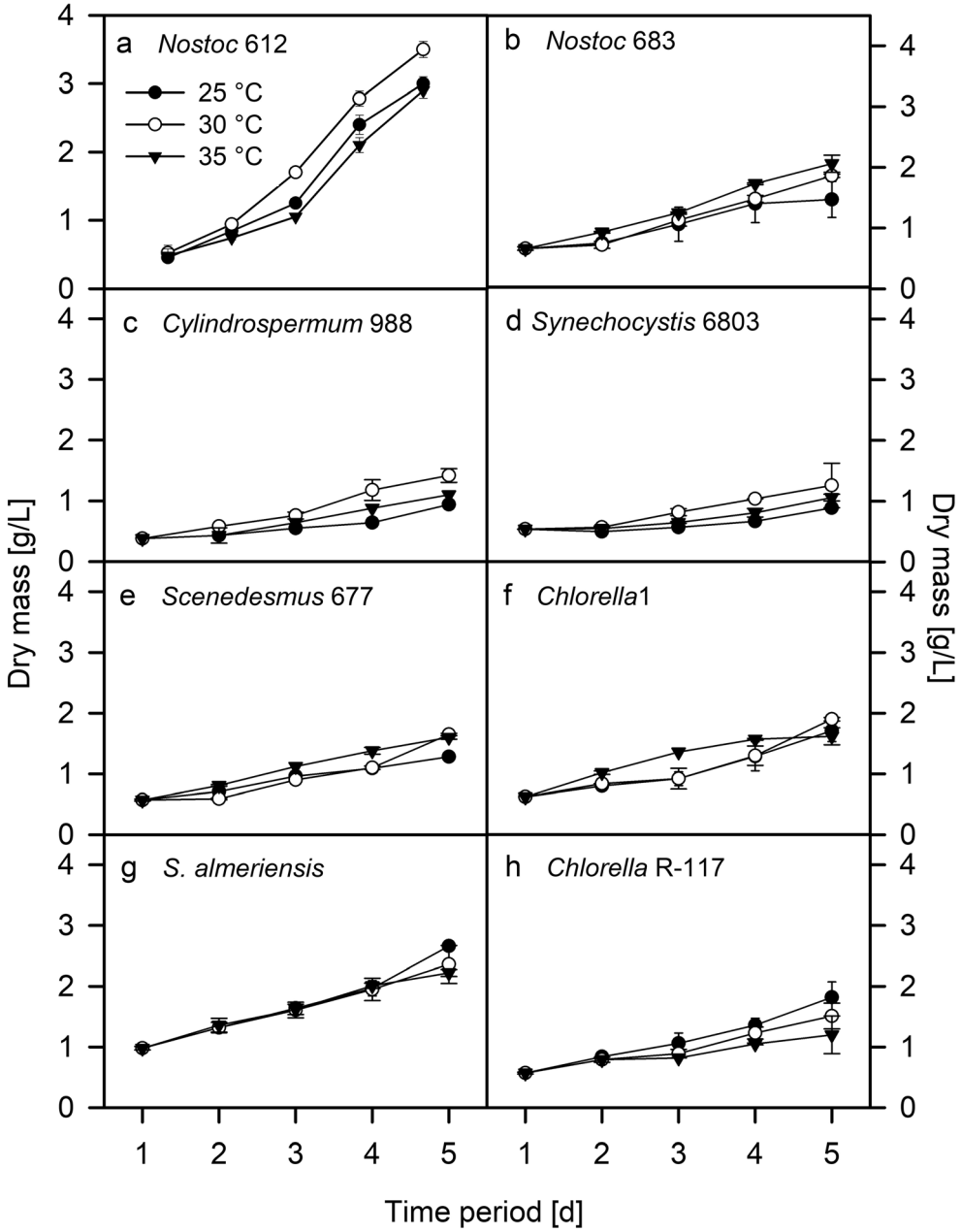

Fig. 5 Growth curves of selected microalgae strains cultivated for 5 days at 25, 30, and 35 °C at the light intensity of 100 μmol/m^2 photon per s. (a) *Nostoc* 612, (b) *Nostoc* 683, (c) *Cylindrospermum* 988, (d) *Synechocystis* 6803, (e) *Scenedesmus* 677, (f) *Chlorella* 1, (g) *S. almeriensis*, and (h) *Chlorella* R-117. Values are presented as a mean ($n = 3$) with SD indicated by error bars

production strain in outdoor large-scale cultivation cascades where the temperatures can be 15 °C in the morning while at midday these reach up to 35 °C. We can speculate that such temperature adaptability can influence the verity of correlation if carried out in laboratory experiments.

Discussion

Our results suggest that rapid measurement of photosynthetic activity can provide a first estimate of optimum growth temperature. In this way, we can quickly evaluate the growth regime for a specific strain using measurements of photosynthetic activity which have been confirmed by regular growth tests and correlate with the growth. For most of the examined strains considered both photosynthetic activity and growth rate tests, the most favourable temperature of 30 °C was found. The optimum temperature may vary depending on the cultivation unit used for trial, especially on the diameter and hence the light path (microalgae can adapt to higher light intensity and higher temperature over time). Due to the variety of cultivation vessel availability, the correlation between photosynthetic activity and growth rate has to be done for each cultivation unit separately.

In our case, we started with a biomass density of ~ 0.5 g/L DM and a light intensity of 100 μmol/m^2 photons per s (d = 3 cm). The optimum growing temperature for *S. almeriensis* was determined to be 25 °C when it was cultivated in 100-mL glass tubes (∅30 × 200 mm). When it was cultivated in a 2-L column with diameter of 60 cm, the light intensity could be increased to 650 umol/m^2 photons per s and the highest growth rate was observed at 35 °C (Sánchez et al. 2008). The microalgae *S. obliquus* was cultured in flasks (LI = 60 μmol/m^2 photons per s) and has a temperature optimum of 31–32 °C, temperatures up to 45 °C did not cause cell death (Hanagata et al. 1992). When we compared the conditions of our experiments and the data found for *S. almeriensis* culture with the data published (Sánchez et al. 2008; Costache et al. 2013), we found that their maxima for biomass productivity was achieved at the temperature of 35 °C at high irradiance (1625 μmol/m^2 photons per s) while at 650 μmol/m^2 photons per s, the highest biomass productivity was found at the temperature of 20 °C. This data suggests that high irradiance plays crucial role in determination of temperature optima. In our experiments, we used a much lower irradiance and the glass tube with the diameter of 30 mm. This might be the source for discrepancy between both data sets.

Concerning the cyanobacteria *Synechocystis* sp., a similar discrepancy was observed where the growth rate was dependent on the light intensity (and thus on the light path in the cultivation unit) as noted by Martínez et al. 2011. In the study of CO_2 biofixation, the experiment was performed in a 1-L column photobioreactor with the diameter of 8 cm. The highest CO_2 biofixation and highest growth were found at the temperature of 35 °C. According to Dauta et al. (1990), the highest growth of *Synechocystis minima* is achieved when the temperature is set to 32 °C.

If we compare a mean activity maximum for all four photosynthetic variables—F_v/F_m, $rETR_{max}$, inflections V_j/V_i, and POE/R for individual strains, these as well as the maxima of growth rate, we cannot see any significant differences ($P < 0.05$) (Table 1). It is important to note that *Chlorella* R-117 is used as a fast-growing production strain in outdoor large-scale cultivation cascades where the temperature can reach up to 35 °C at midday and the culture can adapt to the set conditions without large differences in the growth rates (Converti et al. 2009; Dauta et al. 1990).

The F_v/F_m value is not varying under favourable conditions, but decreases under stress as it reflects a reduction of PSII activity. Temperature regime has been shown to influence not only F_v/F_m but also the oxygen-evolving activity (Bayro-Kaiser and Nelson 2016; Sharkey and Zhang 2010; Valledor et al. 2013). In our experiments, the temperature at which the maximum of F_v/F_m was achieved well correlated with the high growth rate. The same trend was observed when the microalgae strains were characterised by fast fluorescence induction kinetics. The temperature at which the lowest value of V_j and V_i was achieved correlated with the temperature at which the growth rate of microalgae was fastest. For POE/R, these measurements are more sensitive and laborious and take longer time to perform when compared with the much faster fluorescence measurements. We have not found any significant differences ($P < 0.05$) between the optimum temperature obtained from these measurements and the optimum temperature obtained from regular growth tests.

Conclusions

The photosynthetic performance of eight microalgae strains (*Nostoc* 612, *Nostoc* 683, *Cylindrospermum 988*, *Synechocystis* 6803, *Scenedesmus* 677, *Chlorella* 1, *S. almeriensis*, and *Chlorella* R-117) was studied in laboratory experiments through a range of temperatures from 15 to 35 °C. We aimed to develop a fast preliminary test based on photosynthetic measurements (Chl fluorescence and POE/R) in order to estimate a suitable temperature range for growth. Photosynthetic variables—the maximum photochemical yield of PSII, F_v/F_m, $rETR_{max}$, the inflection points V_j/V_i—were chosen to be directly related to the growth. Then, the estimated temperature range was verified in 5-day growth tests, within the results showing rather good correlation without any significant differences ($P < 0.05$). For most of the examined microalgae, a temperature around 30 °C was considered most favourable. Generally, the described procedure can be used as a rapid 1-day pre-screening test to determine suitable growth regime of examined microalgae. Nevertheless, the growth regime in a particular cultivation unit has to be adjusted to the individual microalgae strain.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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