Biophysical Reviews (2022) 14:973–983
https://doi.org/10.1007/s12551-022-00951-9

REVIEW

Chlorophyll fluorescence as a valuable multitool for microalgal biotechnology

Alexei Solovchenko[1,2] · **Alexandr Lukyanov**[1] · **Svetlana Vasilieva**[1,2] · **Elena Lobakova**[1]

Received: 10 March 2022 / Accepted: 26 March 2022 / Published online: 6 April 2022

Abstract

Variable fluorescence of chlorophyll (CF) of the photosynthetic apparatus is an ample source of valuable information on physiological condition of photosynthetic organisms. Currently, the most widespread CF-based technique is represented by recording pulse-amplitude modulated (PAM) induction of CF by saturating light. The CF-based monitoring techniques are increasingly employed for characterization of performance and stress resilience of microalgae in microalgal biotechnology. Analysis of CF induction curves reveals the fate of light energy absorbed by photosynthetic apparatus, the proportions of the energy that have been utilized for photochemistry (culture growth), and heat dissipated by photoprotective mechanisms. Hence CF and its derived parameters are an accurate proxy of the metabolic activity of the photosynthetic cell and the engagement of photoprotective mechanisms. This information is a solid foundation for making decisions on the microalgal culture management during the lab-scale and industrial-scale cultivation. Applications of CF and PAM include the monitoring of stressor (high light, nutrient deprivation, extreme temperatures, etc.) effects for assessment of the culture robustness. It also serves as a non-invasive express test for gauging the effect of assorted toxicants in microalgae. This approach is becoming widespread in ecological toxicology and environmental biotechnology, particularly for bioprospecting strains capable of the destruction of dangerous pollutants such as pharmaceuticals. In the review, we discuss the advantages and drawbacks of using CF-based methods for assessment of the culture conditions. Special attention is paid to the potential caveats and applicability of different variations of CF and PAM measurements for solving problems of microalgal biotechnology.

Keywords Chlorophyll fluorescence · Microalgae · Photoprotection · Monitoring · Industrial cultures · Stress resilience

Introduction

Assessment of photosynthetic organism condition via probing its photosynthetic apparatus (PSA) by recording the variable chlorophyll fluorescence (CF) induction curve (commonly referred to as OJIP curve) became an established approach (Kalaji et al. 2014; Kalaji et al. 2017; Strasser et al. 2004). The advent of affordable devices for measuring CF implementing the principle of pulse-amplitude modulation (PAM) ensured robustness of the results and contributed to the global acceptance of this technique by specialists in plant physiology, ecology, ecotoxicology, and many other fields. A powerful framework for the analysis of OJIP curves known as JIP test has been developed (Strasser et al. 2004). Recent additions to it (Antal et al. 2019) made the whole methodology even more informative and efficient. Detailed accounts on the methodology of CF measurement, analysis, and interpretations of the results can be found in the literature (Stirbet et al. 2014; Strasser et al. 2004). The imaging devices for CF kinetics and endorsement of the CF-based approaches in phenotyping (Jin et al. 2020) made possible another breakthrough in non-invasive express analysis of photosynthetic objects.

The major practical advantage of using CF is that the CF measurements are non-destructive, rapid, and easy to perform. PAM is currently the most widespread technique of CF measurement. It yields valuable information about the fate of the energy of light absorbed by the light harvesting antenna of the PSA reflecting its photochemical utilization and thermal dissipation. Additional parameters have been proposed indicative of diverse processes in photosystem (PS) II such

✉ Alexei Solovchenko
solovchenko@mail.bio.msu.ru

[1] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

[2] Institute of Natural Sciences, Derzhavin Tambov State University, Tambov, Russia

as energy trapping, electron transport, and ΔpH-dependent energy dissipation into heat in the antenna complex (Strasser et al. 2004). These characteristics make CF analysis a particularly attractive method for on-line monitoring of the physiological condition of planktonic (Hanelt 2018) and cultivated (Havlik et al. 2013, 2022) microalgae.

Although the CF-based techniques for higher plants and environmental studies are well established (Porcar-Castell et al. 2021), they are much less elaborated on in the context of microalgal biotechnology and monitoring of the industrial cultures of phototrophs. Still, CF-based approaches for the on-line monitoring of important parameters of algal cultures would be welcomed by microalgal biotechnologists developing processes for the production of high-value bioproducts and biofuels as well as for biomitigation of wastes.

In mass culture of microalgae, understanding the relationships of the acclimatory re-arrangements of the PSA with crucial changes in microalgal cell functioning and its biochemical composition is essential for the estimation of stress intensity and target metabolite accumulation. It is also important for informed and timely adjustment of cultivation conditions. This is important for the cultivated microalgae so they can better cope with the stress, achieve higher productivity, and make the whole cultivation process more robust.

In this review, we briefly summarize the CF-based approaches to sensing the condition of microalgal culture relevant for biotechnology. We do not delve into theoretical details of the CF induction curves analysis since many excellent reviews on this topic exist (Kalaji et al. 2017; Strasser et al. 2004). This review focuses on the applications of CF measurements in the mainstream directions of microalgal biotechnology with the emphasis on the results obtained in recent 10 years in our research group and by our collaborators. Due to the strict word limit, we cannot cite all the relevant works in the field—we apologize for this in advance. At the same time, we did our best to critically consider selection and application of the appropriate CF-based methods for microalgal biotechnologists, discuss their applicability, and highlight their caveats.

Monitoring of the effects in nutrient deficiency and high light

In many microalgal species, environmental stresses such as high PAR irradiance and/or nutrient deficiency induce profound changes in PSA and cell metabolism. Particularly, they trigger accumulation of carbon-rich reserve compounds such as carbohydrates and neutral lipids (Minhas et al. 2016; Vítová et al. 2015). These stress responses of microalgae are frequently exploited in microalgal biotechnology. In frame of this approach, microalgal cultures are exposed to controlled stresses such as depletion of essential nutrients to increase accumulation of the carbon-rich compounds suitable for conversion for biodiesel (Brodie et al. 2017; Vítová et al. 2015) and to trigger the production of hydrogen (Grechanik et al. 2020, 2021). Implementation of this approach requires close monitoring of the culture condition to avoid, on one hand, culture death due to excessive stress and, on the other hand, to achieve the highest possible level of lipid accumulation. Monitoring of CF and derived parameters for this purpose offers distinct advantages over traditional wet biochemical techniques in terms of simplicity and speed. There are also certain benefits in the CF-based methods over optical absorbance-based measurements of cell suspensions. Thus, CF is more sensitive and responsive in revealing the effects of stress during initial stages of the stress exposure where optical absorbance or reflectance-based methods (see, e.g., Solovchenko et al. 2011a, 2011b, 2012) can reliably show the changes in microalgal culture condition only at advanced stages of stressing when these effects are already visible to a naked eye (Havlik et al. 2022). The feasibility of CF-based approach for monitoring high-light and nitrogen-starvation effects on biochemical composition of the microalgae has been proved in a study on a green microalga of biotechnological importance, the mutant of *Parietochloris* producing valuable dihomo-γ-linolenic acid (Solovchenko et al. 2013). A close correlation was found between certain parameters of JIP test, accumulation of carotenoids, lipids, and valuable fatty acids (Fig. 1). The most widely used CF-based parameter is the one reflecting potential maximum photochemical quantum yield of photosystem II, PS II Q_y. It is calculated as $(F_m - F_o) / F_m = F_v / F_m$ where F_o is the minimum level of CF recorded under low intensities of excitation light that do not trigger photosynthesis and F_m is the maximum CF level recorded when all PS II reaction centers are closed by the saturating light pulse (Table 1; see also Maxwell and Johnson 2000).

Analysis of non-photochemical quenching of fluorescence of chlorophyll revealed valuable information about the engagement photoprotection based on thermal dissipation of the absorbed light energy under combined high-light and nitrogen starvation stress. Accordingly, a decline in the non-photochemical quenching (NPQ, Table 1). Therefore, a decline in NPQ magnitude might manifest the overstrain of the photoprotective mechanisms and possibly damage to the cells. Therefore, slow-down of the NPQ build-up on the background of the stress or decline of this parameter was suggested to be a marker of excessive stress exposure with a potentiality to impair the culture productivity if no corrective action will be taken. Technically, using the NPQ parameter as a proxy to NPQ level in the cell is simpler than other parameters related to quenching since it does not require the knowledge of the minimum CF intensity, F_o (Table 1, see also Tian et al. 2019). The use of NPQ parameter as an

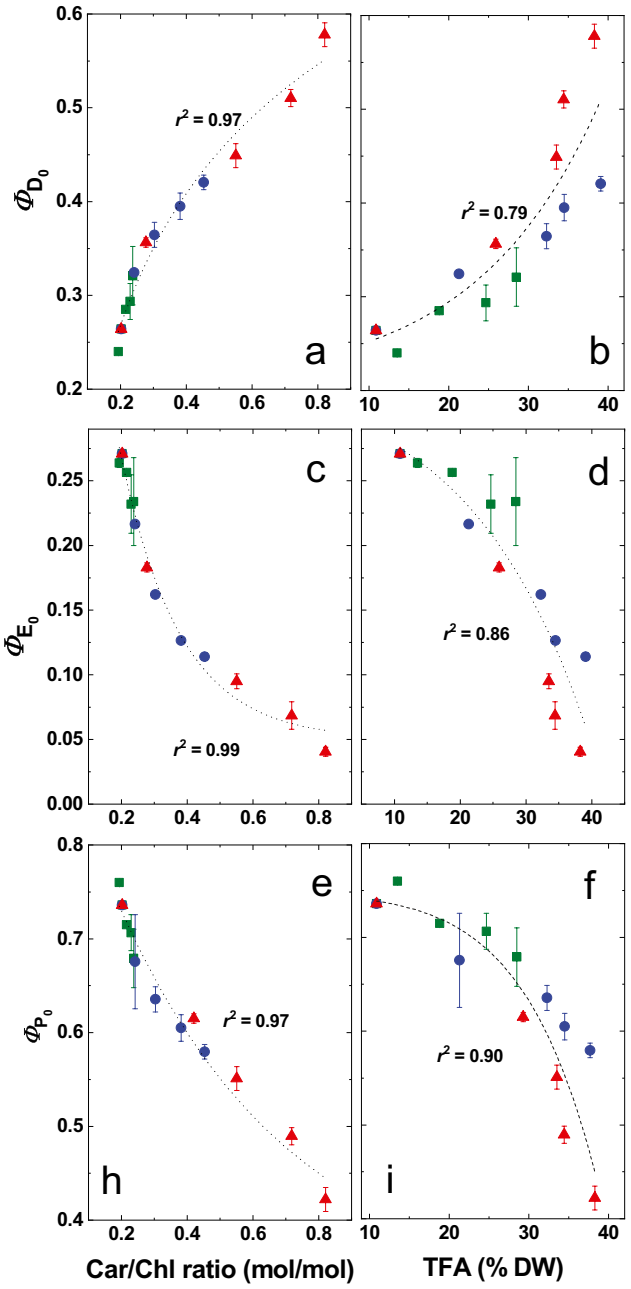

Fig. 1 Relationships of quantum yields of energy dissipation (**a**, **b**) and electron transport (**c**, **d**) and maximal quantum yield of primary photochemistry (**e**, **f**) with carotenoid-to-chlorophyll ratio (**a**, **c**, **e**) and total fatty acid DW percentage (**b**, **d**, **f**) in nitrogen-deprived P127 mutant of *P. incisa* cultivated under low (squares), medium (circles), or high (triangles) light. Reprinted from Solovchenko et al. (2013) with permission from Elsevier. For explanation of the parameters of JIP test, see Stirbet et al. (2014) and Strasser et al. (2004) and Table 1

indicator of metabolic status of microalgal cell is further discussed below.

Simple approaches such as those based only on PS II Q_y can be used as a rapid gauge of nutrient deficiency (Juergens et al. 2015). At the same time, they can suffer from insufficient selectivity and poor sensitivity since they rapidly decline at the onset of stress and show little variation thereafter. Thus, monitoring of PS II Q_y allowed to reliably detect the onset only of nitrogen-deficiency stress in bloom-forming cyanobacterium *Microcystis aeruginosa* UTEX LB 3037 but it was impossible to discern the stresses caused by the lack of other nutrients such as P or Fe (Perri et al. 2021). Notably, the low selectivity this approach did not depend on dark adaptation (Perri et al. 2021). Moreover, one should be aware that PS II Q_y never reaches its formal limit (unity) due to competition of different dissipative pathways in PS II (Kalaji et al. 2014; 2017; Strasser et al. 2004). Normally, it does not exceed 0.75 for rapidly dividing non-stressed cultures of eukaryotic microalgae; in the case of cyanobacteria is generally lower (around 0.4–0.5). The PS II Q_y of stressed cultures can drop almost to zero (when the minimum and the maximum CF levels are close to each other). One should be aware that unusually high values of PS II Q_y can be caused by the noise exerting a dominant contribution when the measured intensity of CF is around or below the sensitivity limit of the PAM detector. In case of getting PS II Q_y values above 0.75, it is strongly recommended to double check the experimentally measured F_m and F_o values used for the calculation of PS II Q_y (Table 1).

To achieve the highest productivity both in terms of biomass amount and the content of target metabolites in the biomass, two-phase batch cultivation approach is used. During the first phase, the maximum cell division rate (and accumulation of biomass) in the nutrient-replete is achieved. The cells are then subjected to a stress (see above) to induce the synthesis of the target metabolites. The wet chemical assay of the residual level of the nutrients in the cultivation medium is usually costly, laborious, and hazardous to the environment. Instead of it, on-line monitoring of JIP test parameters of the culture in the photobioreactor allows to pinpoint the moment of complete nitrate exhaustion in the cultivation medium (Plyusnina et al. 2020). It was manifested by a transient increase in the efficiency of PS II when the number of PS II reaction centers declined with a simultaneous increase of the effective cross section of the light-harvesting antenna per reaction center. However, prolonged nitrogen starvation induced typical acclimatory changes in the PSA described above.

Even more powerful approach to analyze the fluorescence transients implies deconvoluting them into exponentials approximately corresponding to the OJIP phases using spectral multiexponential approximation (SMEA) developed by Plyusnina et al. (2020). SMEA allows one to detect visually indistinguishable phases of induction curves, presence, or disappearance of individual phases reflecting the changes of culture physiological condition (exponential growth, stress onset, and stress acclimation). SMEA analysis was successfully applied to OJIP curve analysis in *Chlamydomonas*

Table 1 The JIP test parameters frequently used in monitoring of microalgal cultures (Antal et al. 2009; Strasser et al. 2004)

Parameters	Description	Comments	Typical value ranges and trends of change
F_O, F_j, F_{300}, F_m (F_O', F_m')	Fluorescence yield at the points O (minimal fluorescence, when all PS II reaction centers, RC, are open), J (2 ms), at 300 μs (K-step), and at the point of the maximum of OJIP, M, when all PS II RC are closed. (In the parentheses are the same parameters recorded under light-adapted conditions)	Characteristic points of OJIP curve used for deriving of the JIP test parameters	Seldom used per se since the absolute CF values depend on chlorophyll content of the samples, the PAM machine used, and the settings made
PS II $Q_y = \varphi_{P_0} == \frac{F_m - F_O}{F_m} = \frac{F_v}{F_m}$	Maximum potential quantum yield of primary photochemistry in photosystem II	The most widely used parameter sensitive to many stresses. Under severe stress can lose sensitivity. Requires dark adaptation of the sample	0.75–0.6 for non-stressed exponentially growing microalgal cultures (0.5–0.4 for cyanobacteria). Tends to decline upon the onset of stationary phase and/or under stress. Increases in dense cultures due to self-shading of the cells
$PSIIQ_y' = \varphi'_{PO} = \frac{F_m' - F_O'}{F_m'} = \frac{F_v'}{F_m'}$	Operational or real quantum yield of primary photochemistry in photosystem II	Measured under light-adapted conditions. Gives realistic information about the condition of the cell and acclimation state of the PSA under actual conditions	PS II Q_y' is lower than PS II Q_y measured in same dark-adapted samples. The higher the intensity of the actinic light (e.g., solar light impinging on the outdoor photobioreactor), the higher the decline of PS II Q_y' as compared with the corresponding PS II Q_y values
$NPQ = \frac{F_m}{F_m'} - 1$	A non-photochemical quenching parameter according to Stern–Volmer	A proxy to the intensity of thermal dissipation of the light energy absorbed by the PSA. Handy since it does not require F_o measurement. Indicative of stress intensity, metabolic quiescence, and the onset of damage	It is normally well below 0.5 for non-stressed exponentially growing microalgal cultures. Under mild stress increases to 1–1.5, can be as high as 4 under strong stress (e.g., combined high light and nitrogen starvation)
$\psi_0 = 1 - \frac{F_J - F_O}{F_M - F_O}$	The probability of electron transfer from PS II to PQ pool	Useful for monitoring of changes in electron transport in chloroplast ETC	Typically follows the same pattern as PS II Q_y but can remain relatively high under mild stress when NPQ is engaged and/or in the presence of a strong metabolic sink of the photosynthates
$M_0 = 4\frac{F_{300} - F_O}{F_M - F_O}$	An approximation of the initial slope of OJIP attributed to Q_A reduction	A proxy to the rate of primary quinone acceptor reduction	Increases rapidly when the electron carriers of the chloroplast electron transport chain get reduced (e.g., under stress)
$\phi_{D_0} = \frac{F_O}{F_M}$	Quantum yield of energy dissipation	A proportion of the absorbed light energy that is dissipated to heat for protection of the PSA from photodamage	Its pattern of change is opposite to that exhibited by PS II Q_y
$\phi_{E_0} = \Psi_0\left(1 - \frac{F_O}{F_M}\right)$	Quantum yield of electron transport	A proportion of the absorbed light energy that is used to drive the electron transport in the chloroplast ETC	Displays the change pattern qualitatively similar to that of PS II Q_y
$\mathrm{ABS/RC} = M_0 \cdot \frac{1}{V_J} \cdot \frac{1}{\varphi_{P_0}}$	Absorbed energy flux per reaction center (RC)	Amount of the absorbed light energy per single RC. This parameter increases often under adverse conditions due to decline in the number of functional RCs	Usually increases under adverse conditions damaging to the reaction centers. In this case reflects the flow of the same or increasing amount of energy through a declining number of active reaction centers
$\mathrm{PI_{ABS}} = \mathrm{RC/ABS}\left(\frac{\varphi_{P_0}}{1-\varphi_{P_0}}\right)\left(\frac{\psi_0}{1-\psi_0}\right)$	Performance index expressed on the light absorption basis	A combination parameter reflecting overall performance of the PSA. Sensitive even to mild stresses	In non-stressed exponentially growing microalgal cultures, it is typically around 0.5 or higher. Under stress, rapidly drops to almost zero

reinhardtii starving for sulfur (Antal et al. 2019), as well as *Chlorella vulgaris* starving for nitrogen (Plyusnina et al. 2020). An important advantage of SMEA is that it allows to detect the onset of stress during continuous monitoring of the culture conditions. The advent of photobioreactors fitted with automatic PAM fluorometers together with the development of special software packages such as pyPhotoSyn with the support of the necessary calculations facilitates implementation of the advanced CF express analysis in real time.

Salinity

Salinity stress, also in combination with high light and nutrient limitation, is frequently used to control the biosynthesis of reserve lipids enriched in saturated fatty acids suitable for the production of biodiesel in marine microalgae such as *Nannochloropsis oceanica* (Vonshak et al. 2020). On the contrary, valuable polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (C20:5, EPA) are accumulated in chloroplast lipids under favorable conditions conductive for cell division and expansion of light-harvesting antenna in the PSA of the microalga (Solovchenko et al. 2014a). The variation of the salinity in the studied range had only a slight effect on NPQ: even high salinities (above 27 g/L) triggered a detectable increase of NPQ (Martínez-Roldán et al. 2013), whereas the increase in NPQ induced by nitrogen deprivation was considerably higher in this species. Monitoring of NPQ revealed the differential engagement of photoprotective mechanisms of *N. oceanica* in response to elevated salinity, high light (Solovchenko et al. 2014a), and after addition of exogenic photosensitizer Rose Bengal and methyl viologen promoting oxidative stress (Vonshak et al. 2020) on the background of elevated salinity. Rapid light curve (RLC) based of recording PS II Q_y as a function of irradiance (Ihnken et al. 2010) was a good proxy of the photosynthetic capacity of the culture. Collectively, this information allows for timely taking of informed culture management decisions to steer the cultivation process to production of the biomass suitable either for biodiesel or valuable PUFA extraction.

Chilling tolerance

Certain species of biotechnologically important microalgae respond to chilling stress by increasing PUFA content in their membrane lipids. This response is important to keep the fluidity of the lipid bilayer at a level ensuring correct functioning of the membrane apparatus including the proteins constituting the PSA, at a low temperature. The methods based on CF registration were suitable for monitoring of chilling effects on *Lobosphaera incisa*—a green microalga that accumulates high levels of the valuable omega-6 PUFA arachidonic acid (ARA, C20:4). In our recent studies, JIP test has been applied to elucidate the physiological significance of the accumulation of ARA (Kugler et al. 2019; Zorin et al. 2017). Thus, the mutant of *L. incisa* P127 deficient in ARA biosynthesis hence featuring impaired the homeoviscous adaptation response showed increased NPQ levels when exposed to chilling temperatures (Zorin et al. 2017). We hypothesized that the high levels of NPQ typical of P127 mutant were likely associated with the lowered abundance of polyunsaturated fatty acids in membrane lipids and changes of the activity of violaxanthin de-epoxidase (VDE). The activity of VDE depends on the unsaturation level of galactolipids, along with their ratio, which are necessary for violaxanthin solubilization in lipid phase (Garab et al. 2016).

Further studies of the psychrotolerance of *L. incisa* have been carried out with the strain IPPAS C2047. Comparative analysis of the JIP test parameters under optimal and chilling (0°) temperatures revealed the mode of photoprotection employed by this microalga under the chilling conditions (Ptushenko et al. 2021). A remarkable decrease in redistribution of energy in PS II and a decline in the efficiency of photosynthetic electron transport were recorded. Specifically, a decline in photochemical activity as well as a significant increase in the thermal dissipation of the absorbed light energy in the light-harvesting antenna took place in the microalgal cells incubated at 0°. At the same time, the pigment apparatus including violaxanthin cycle xanthophylls remained virtually unchanged according to the HPLC analysis. The observed effects were ascribed to the dramatic increase in ARA and expression of LhcSR, a protein responsible for quenching of excitation in the photosynthetic antenna. Collectively, the CF-based probing of microalgal cell condition confirmed that *L. incisa* under short-term chilling relies mostly on LhcSR-dependent NPQ to protect its PSA; longer chilling results in the engagement of additional acclimatory mechanisms (Ptushenko et al. 2021).

Monitoring of wastewater treatment efficiency

Microalgae as photoautotrophic organisms produce oxygen which accelerates the degradation of diverse pollutants in the treated wastewater (Muñoz and Guieysse 2006). An added benefit of this approach is the production of microalgal biomass which can be used as raw material in many applications such as production of biofuel, value-added feed and food additives, and fertilizers (Mata et al. 2010; Skjånes et al. 2012). Therefore, biotreatment with microalgae rapidly emerges as a promising alternative to the conventional wastewater treatment technologies. However, close monitoring of the culture condition is a prerequisite for efficient treatment of waste streams with microalgae. Failure to notice

the signs of culture intoxication by excessive pollutant concentration or inhibition of photosynthesis by a suboptimal cultivation parameter can lead to a rapid culture death and hence to a complete loss of the efficiency of the biotreatment facility. Timely and informed decisions on the adjustment of illumination conditions, the rate of wastewater addition, and on the time for biomass harvesting are necessary to make this biotechnology viable.

CF-based monitoring of microalgae offers a viable alternative to the conventional methods of biochemical analysis along with the advantages of high sensitivity and affordability (Havlik et al. 2013, 2015, 2022). An illustrious example of its feasibility is comprised by monitoring of the condition of *Chlorella sorokiniana* culture in an annular PBR employed for bioremediation of alcohol distillery wastewater (Solovchenko et al. 2014b) representing a serious environmental hazard (2009). Online measurements of PS II Q_y with a custom-made PAM sensor provided ample information about the acclimation state of the culture and the rate of organic pollutant bioremoval in a high-density semi-batch PBR (Fig. 2). The CF-based monitoring method was more responsive in comparison with monitoring of the culture via optical density allowing for swift corrective actions restoring the optimal pH and pollutant load rate of the culture. Overall, a large body of evidence supports the suitability of the CF-based monitoring. This approach also holds promise for the development of automated processes for combined secondary and tertiary treatment of waste streams that would be simpler and more affordable than currently used activated sludge-based processes.

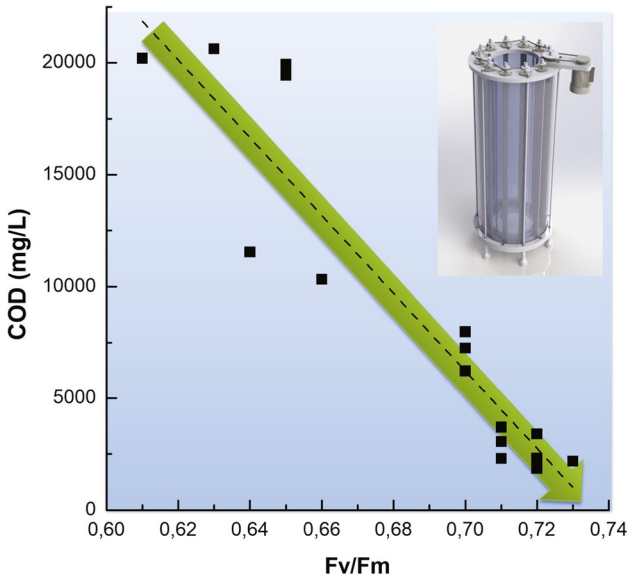

Fig. 2 Relationships between maximum PS II efficiency, F_v/F_m and organic pollutant content (expressed as chemical oxygen demand, COD) of pH-adjusted alcohol distillery wastewater during cultivation of *Chlorella sorokiniana* in the photobioreactor. The data for three representative sequential cycles of semi-batch cultivation (400 h total cultivation time) are shown. Adapted from Solovchenko et al. (2014b)

Monitoring of carotenogenic response

Certain algal species called carotenogenic microalgae respond to environmental and other stresses with massive accumulation of carotenoids (Solovchenko and Neverov 2017; Solovchenko 2013). An illustrious example of carotenogenic microalgae is the chlorophyte *Haematococcus pluvialis*, an ample source of natural astaxanthin—one of the most biotechnologically significant pigments with a plethora of applications and beneficial effects on human health (Kanwugu et al. 2021).

One of the bottlenecks for the microalgae-based biotechnologies for production of natural astaxanthin and other high-value carotenoids is comprised by a high mortality of cells during induction of the carotenogenic response (Minyuk et al. 2019; Minyuk et al. 2016). Another problem is finding the optimal mode of the microalgal culture supply with the nutrients and CO_2. As in other microalgae, insufficient CO_2 supply limits culture productivity whereas its oversupply exerts deleterious effects on cell division and hence on its productivity. Our recent studies (Chekanov et al. 2016, 2017) showed that the changes in primary photochemistry, electron flow at the acceptor side of PS II, and NPQ can be characterized using PAM chlorophyll fluorimetry. The obtained results shed light on the stress-induced onset of carotenogenesis and on the overall culture condition. The CF monitoring allowed to discover a transient up-regulation of cell metabolism (and active energy-dependent photoprotective mechanisms) during stress-induced formation of metabolically quiescent, astaxanthin-rich aplanospores by *H. pluvialis*. We hypothesized that the transient stimulation of photochemical utilization of light energy might be necessary to satisfy the metabolic demand of energy and photosynthates necessary for the astaxanthin and fatty acid biosynthesis. Later, a transition takes place from regulated, energy-dependent quenching qNPQ activated by the actinic light to non-regulated irradiance-independent quenching, qNO (Kramer et al. 2004). The non-relaxing (constitutively operating) components of NPQ also emerged along with the build-up of optical shielding by astaxanthin ample in the mature resting aplanospores (haematocysts). These processes were accompanied by an overall decline in NPQ which is not typical to phototrophs under stress but represents an informative manifestation of the carotenogenic response (Chekanov et al. 2019). From the practical standpoint, the CF-based parameters such as very low PS II Q_y and qNPQ as well as significant qNO can be employed as manifestations of haematocyst maturation. This is important since

mature haematocysts can be further stressed for boosting astaxanthin accumulation without the risk of increased cell mortality.

Flashing light effects

Growing microalgal cultures at a high cell density is frequently problematic since the incident light is rapidly attenuated by superficial layers of the culture. As a result, the cells dwelling below the few millimeter-thick superficial layer remain effectively in darkness most of the time. To circumvent this limitation, illumination of the cultures with high-intensity flashing light was suggested (Abu-Ghosh et al. 2015, 2018). This illumination mode provides enough energy to drive photosynthesis in deep layers of a dense culture but does not impose the risk of photodamage inevitable in case of continuous intensive illumination. To assess the effects of different combinations of irradiance and duty cycle, registration of the CF parameters such as PS II Q_y and NPQ proved to be efficient. Indeed, photosynthesis and growth in *Dunaliella salina* were significantly enhanced under flashing light with a light–dark cycle tailored for the minimal activation of the violaxanthin cycle in comparison with cultures grown under constant illumination of the same time-integrated photon dose. Thus, the induction of NPQ was significantly slower in the flashing light-acclimated cells compared with those acclimated to continuous illumination. The authors concluded that a slower activation of NPQ takes place under balanced flashing light, when each flash is followed by a dark period long enough for utilization of the photons absorbed during the flash at a given irradiance (Abu-Ghosh et al. 2015).

Accordingly, the activation of thermal dissipation of the absorbed light energy manifest by an increase in NPQ played an important role in modulation photosynthesis observed in *D. salina* grown under flashing light. The balanced parameters of flashing light conductive for efficient photosynthesis and growth of *D. salina* correlated with a slow and limited activation of the VC apparent as low-to-moderate NPQ. By contrast, suboptimal flashing light parameters lead to a rapid build-up of NPQ and impaired photosynthesis and growth.

The feasibility of monitoring of the effects of flashing light with the CF-based techniques is based on tight relationship between the degree of the engagement of photoprotective mechanisms under flashing light and synchronization between flashing light duty cycle and light utilization by the microalgal cell. Therefore, NPQ level was suggested as a reliable criterion for the selection and fine-tuning of flashing illumination parameters for the cultivation of microalgae.

Deleterious and beneficial effects of CO_2 enrichment

Photosynthetic microorganisms require CO_2 as a substrate for their autotrophic growth, and therefore, they are thought to be a promising vehicle for biomitigation of technogenic CO_2 emissions (Van Den Hende et al. 2012). At the same time, excessive enrichment of the culture with CO_2 can cause deteriorative effects due to acidification of the culture medium by the carbonic acid forming upon dissolving of the gaseous CO_2 (Solovchenko and Khozin-Goldberg 2013). Development of microalgae-based technologies for sequestration of CO_2 from flue gases requires a deep understanding of the mechanisms of microalgal tolerance to elevated CO_2. In addition to that, such biotechnologies require the method of on-line monitoring of the photosynthetic performance of the culture allowing to balance the CO_2 input against assimilatory capacity of the microalgal cells and other cultivation parameters such as illumination intensity and mixing rate. Monitoring of the CF reflecting the condition of PSA is a plausible candidate technique for accomplishing this goal.

The build-up of high CO_2 tolerance in a symbiotic chlorophyte *Desmodesmus* sp. IPPAS S-2014 was found to be related, in particular, to a high level of NPQ which, according to the results of JIP test, was induced in response to high (20%, by volume) CO_2 (Solovchenko et al. 2015) and exacerbated by an additional stressor (nitrogen deprivation, see Ptushenko and Solovchenko 2016). Obviously, the high NPQ in this case lowering the light energy capture and ensuring safe dissipation of light energy potentially harmful under the stressful conditions. Interestingly, in contrast to free-living microalgae, the atmospheric CO_2 level and corresponding limitation by inorganic carbon (especially significant at high irradiances) seem to be stressful to *Desmodesmus* sp. IPPAS S-2014 judging from a slightly increased PS II Q_y and elevated NPQ level.

Analysis of OJIP curves, especially in terms of NPQ-related changes, revealed significant details about the effects of CO_2 in the carotenogenic microalga *H. pluvialis* B-2018. Vegetative cells of this microalga are relatively tolerant to slightly elevated CO_2 levels (below 10 vol. %) but adding 20% of CO_2 to the sparging gas mixture rapidly decreases PS II Q_y (Chekanov et al. 2017). Interestingly, increasing the CO_2 to 5% was beneficial to the photosynthetic performance of the culture and viability of the cells, likely due to a higher sink capacity of the cell in the absence of inorganic carbon limitation.

Notably, the sensitivity of PS II Q_y to the intensity of a stress can be impaired, as was demonstrated by Chekanov et al. (2017). Therefore, monitoring of additional

parameters reflecting the capability of the chloroplast ETC of intersystem electron transfer was required to reliably the phototrophic cell condition taking into account the redox state of the plastoquinone pool. Thus, the parameter Ψ_o (Table 1) is a proxy to the probability of electron transport beyond the primary PS II quinone acceptor Q_A. Low values of Ψ_o manifest overreduction of electron carriers in the ETC. The analysis of Ψ_o confirmed the beneficial effect of 5% CO_2 in red *H. pluvialis* cells. This conclusion was supported by an increase in turnover of the primary PS II quinone acceptor (Q_A) indicative of a higher rate of the photosynthetic electron transport. In view of current findings, a combination of the selected JIP test parameters (PS II Q_y, NPQ, Ψ_o) can be recommended for on-line monitoring of elevated CO_2 effects in microalgae.

Gauging of hazardous micropollutant effects

Pharmaceuticals such as antibiotics, non-steroid anti-inflammatory drugs, natural, and synthetic hormones are hazardous micropollutants (HMP) imposing a serious threat to the environment (Fallah et al. 2021). Bioprospecting of tolerant strains capable of HMP destruction is an important goal in the development of microalgal biotechnologies for biotreatment of HMP-loaded waste streams. To isolate suitable candidate organisms, many isolates need to be tested. Fortunately, time-consuming and labor-intensive trials of the microalgal isolates can be, at least at the initial phase, replaced with a non-invasive technique based on CF monitoring. Since the PSA of microalgae exhibits distinct responses to HMP contamination (Grzesiuk et al. 2018), PAM-based techniques for biotoxicity assay become increasingly widespread. Recently, we tested the suitability of PAM imaging of Chl variable fluorescence for the assay of HMP effects on microalgae and cyanobacteria in well plate format (Solovchenko et al. 2022). This approach allowed for automated simultaneous processing of several samples, although certain precautions should be taken. Still, this technique is convenient for kinetic experiments offering a considerable time saving. The imaging PAM-based technique based on the PS II Q_y and NPQ parameters better resolved the rapid changes in the microalgal cell condition in comparison with traditional biochemical assays like chlorophyll monitoring. Importantly, imaging PAM was also sensitive enough to discern the effects of low concentration of the HMP typical for waste streams and environment.

At the same time, some precautions were underlined essential for obtaining of correct screening results. Since the PAM imaging measurements of microalgal cultures are conducted in well plates, the well plates should be sealed to avoid rapid evaporation of water though CF measurements are less prone to errors due to evaporation. One should also bear in mind that the inoculation of well plates is a stressful manipulation per se so it can trigger a decline of Q_y in control samples. Therefore, it is better to start the measurements after an acclimation period (normally around 24 h). The illumination intensity during the incubation of the well plates should not be too high to avoid high-light stress but high enough provide sufficient energy for microalgae. Finally, the imaging plates should be used to enable selective detection of the CF emitted from individual wells.

Effects of microalgal cell immobilization

Immobilization of microalgae is becoming increasingly widespread in production of microalgal biomass and value-added metabolites derived from it, and waste treatment. Immobilized cultures are often preferred for their higher biomass productivity, stress resilience, cell retention, and absence of mixing requirement (Mallick 2006; Tsygankov and Kosourov 2014). As in suspended cell cultivation, immobilized cells should be monitored for their physiological condition and photosynthetic performance to ensure the stability of the culture. For microalgal cells immobilized on the surface of polymeric cell carriers, this goal was successfully accomplished with PAM imaging (Vasilieva et al. 2021). In particular, this method is very helpful in assessments of biocompatibility of cell carrier materials as well as of performance of new biohybrid materials containing immobilized phototrophic cells (Vasilieva et al. 2021). Importantly, the imaging approach allows to take into account the possible non-uniformity of PSA parameters of the cells distributed over a large biohybrid material sample surface which is difficult to achieve with normal "point-based" CF measurements. Acclimation of the cells after immobilization is normally manifested by a transient decline in PS II Q_y followed by its gradual recovery (and an opposite trend exhibited by NPQ).

Concluding remarks

In this review, we briefly summarized biotechnologically relevant applications of CF-based methods for monitoring of microalgal cultures. These methods became a welcome addition to the traditional wet methods of biochemical analysis due to their distinct advantages of simplicity, sensitivity, robustness, and high throughput of measurements. Implementation CF-based online monitoring methods will improve the robustness of biotechnologies in which microalgae are mass cultivated on the edge of potential damage by stressful conditions necessary for obtaining the highest possible yield of target bioproducts. Of special interest is

CF-based monitoring of cultures in combination technologies such as astaxanthin production and CO_2 bio-sequestration by *H. pluvialis* cultivation. Another possible direction is the boosting of lipid production with combined exposure of the cultures to nutrient deprivation and high light where the microalgae are especially vulnerable to photooxidative damage.

However, successful application of the CF-based monitoring of the cultures of phototroph microorganisms depends, as in the case of higher plants, on the correct experimental design and measurement conditions. In particular, one needs to be aware of applicability of different CF measurement techniques as well as of meaning of various parameters, e.g., the parameters of JIP test. The parameters most frequently used in the monitoring of microalgal cultures are summarized in Table 1 (although this list is by no means exhaustive). General rules of thumb of the CF analysis in higher plants were nicely summarized before (Goltsev et al. 2016; Kalaji et al. 2014, 2017; Maxwell and Johnson 2000). Most of these guidelines are valid also for microalgal cultures although some peculiarities exist. The minimalistic checklist of precautions which should be observed to ensure the correct information output of the CF measurement of microalgal cultures includes the following.

(1) For each independent experiment (cultivation batch), there should be a separate untreated or unstressed control. It will allow for correct comparison of the results across experiments since slight differences in the conditions are inevitable even in the sequential experiments intended to be carried out under the same conditions.
(2) Correct intensities of measuring and saturating light fluxes should be chosen. That is, the light use for the F_o recording should not trigger photosynthesis and that used for F_m capture should reliably saturate the PSA but induce no photodamage. Ideally, each of them should be verified independently (by oxygen evolution light curve and CF induction curve shape monitoring), especially if no literature reference is available for this organism and experiment setup.
(3) The cell density of the culture samples should be adequate to the light fluxes used: all the cells in the sample should be irradiated by roughly the same flux. Failure to do so (e.g., because of mutual shading of the cells) results in misleading measurements since the shaded cells will not be exposed to the saturating light no matter how intense the light is impinging on the sample. The same reasoning urges to avoid a sizeable cell sedimentation during the measurements and to ensure uniform irradiation of the sample in the imaging PAM measurements. Also beware of biofouling in flow-through PAM measurement systems.
(4) Choose informative parameters of JIP test. For example, dark-adapted measurements of PS II Q_y might not always reflect the difference in stress resilience of the cultures. On the contrary, it can be readily observed from the operational (actinic light-adapted) measurements. Combination parameters such as performance index, P_i (Table 1), can be more sensitive than individual quantum yield- or flux-related parameters. In all cases, confront the selection of the JIP test parameters with the experimental design and the goal of the experiment.

To conclude, the tight integration of photosynthesis with all other processes in phototrophic cell makes probing of PSA via CF measurements a powerful method of the assessment of microalgal cultures. It provides ample information of vast practical relevance for microalgal biotechnologists and gives the unprecedented opportunities for knowledge-based on-line culture measurements and control. At the same time, a deep understanding of the PSA regulation and the connections between the basic stress responses and the observable changes in the CF parameters are required for successful using of these promising techniques. Further studies of structure and function responses of PSA will undoubtedly lead to the development of more advanced knowledge-driven microalgal biotechnologies.

Author contribution All authors contributed to the study conception and design. The first draft of the manuscript was written by Alexei Solovchenko and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Financial support of Russian Science Foundation (project 21–74-20004) is gratefully acknowledged.

Declarations

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

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Abu-Ghosh S, Dubinsky Z, Banet G, Iluz D (2018) Optimizing photon dose and frequency to enhance lipid productivity of thermophilic algae for biofuel production. Bioresource Technol 260:374–379. https://doi.org/10.1016/j.biortech.2018.03.110

Abu-Ghosh S, Fixler D, Dubinsky Z, Solovchenko A, Zigman M, Yehoshua Y, Iluz D (2015) Flashing light enhancement of photosynthesis and growth occurs when photochemistry and

photoprotection are balanced in *Dunaliella salina*. Eur J Phycol 50:469–480. https://doi.org/10.1080/09670262.2015.1069404

Antal T (2018) The metabolic acclimation of *Chlamydomonas reinhardtii* to depletion of essential nutrients: application for hydrogen production. In: (Seibert M, Torzillo G, eds) Microalgal hydrogen production: achievements and perspectives. Royal Society of Chemistry 16 235–264. https://doi.org/10.1039/9781849737128-00235

Antal T, Konyukhov I, Volgusheva A, Plyusnina T, Khruschev S, Kukarskikh G, Goryachev S, Rubin A (2019) Chlorophyll fluorescence induction and relaxation system for the continuous monitoring of photosynthetic capacity in photobioreactors. Physiol Plant 165:476–486. https://doi.org/10.1111/ppl.12693

Antal T, Matorin D, Ilyash L, Volgusheva A, Osipov V, Konyuhov I, Krendeleva T, Rubin A (2009) Probing of photosynthetic reactions in four phytoplanktonic algae with a PEA fluorometer. Photosynth Res 102:67–76. https://doi.org/10.1007/s11120-009-9491-6

Brodie J et al (2017) The algal revolution. Trends Plant Sci 22:726–738. https://doi.org/10.3389/fpls.2020.609993

Chekanov K, Lukyanov A, Boussiba S, Aflalo C, Solovchenko A (2016) Modulation of photosynthetic activity and photoprotection in *Haematococcus pluvialis* cells during their conversion into haematocysts and back. Photosynth Res 128:313–323. https://doi.org/10.1007/s11120-016-0246-x

Chekanov K, Schastnaya E, Neverov K, Leu S, Boussiba A, Zarka A, Solovchenko A (2019) Non-photochemical quenching in the cells of the carotenogenic chlorophyte *Haematococcus lacustris* under favorable conditions and under stress. Biochim Biophys Acta (BBA)-General Subjects 1863:1429–1442. https://doi.org/10.1016/j.bbagen.2019.05.002

Chekanov K, Schastnaya E, Solovchenko A, Lobakova E (2017) Effects of CO_2 enrichment on primary photochemistry, growth and astaxanthin accumulation in the chlorophyte *Haematococcus pluvialis*. J Photochem Photobiol b: Biol 171:58–66. https://doi.org/10.1016/j.jphotobiol.2017.04.028

Fallah Z et al (2021) Toxicity and remediation of pharmaceuticals and pesticides using metal oxides and carbon nanomaterials. Chemosphere 275:130055. https://doi.org/10.1016/j.chemosphere.2021.130055

Garab G, Ughy B, Goss R (2016) Role of MGDG and non-bilayer lipid phases in the structure and dynamics of chloroplast thylakoid membranes. Subcell Biochem 86:127–157. https://doi.org/10.1007/978-3-319-25979-6_6

Goltsev VN et al (2016) Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. Russ J Plant Physiol 63:869–893. https://doi.org/10.1134/S1021443716050058

Grechanik V, Romanova A, Naydov I, Tsygankov A (2020) Photoautotrophic cultures of *Chlamydomonas reinhardtii*: sulfur deficiency, anoxia, and hydrogen production. Photosynth Res 143(3):275–286. https://doi.org/10.1007/s11120-019-00701-1

Grechanik V, Naidov I, Bolshakov M, Tsygankov A (2021) Photoautotrophic hydrogen production by nitrogen-deprived *Chlamydomonas reinhardtii* cultures. Int J Hydrogen Energy 46(5):3565–3575. https://doi.org/10.1016/j.ijhydene.2020.10.215

Grzesiuk M, Spijkerman E, Lachmann SC, Wacker A (2018) Environmental concentrations of pharmaceuticals directly affect phytoplankton and effects propagate through trophic interactions. Ecotoxicol Environ Safety 156:271–278. https://doi.org/10.1016/j.ecoenv.2018.03.019

Hanelt D (2018) Photosynthesis assessed by chlorophyll fluorescence. In: Häder D-P, Erzinger GS (eds) Bioassays. Elsevier, pp 169–198. https://doi.org/10.1016/B978-0-12-811861-0.00009-7

Havlik I, Beutel S, Scheper T, Reardon KF (2022) On-line monitoring of biological parameters in microalgal bioprocesses using optical methods. Energies 15:875. https://doi.org/10.3390/en15030875

Havlik I, Lindner P, Scheper T, Reardon KF (2013) On-line monitoring of large cultivations of microalgae and cyanobacteria. Trends Biotechnol 13:406–414. https://doi.org/10.1016/j.tibtech.2013.04.005

Havlik I, Scheper T, Reardon K (2015) Monitoring of microalgal processes. Advances in biochemical engineering/biotechnology. Springer, Berlin Heidelberg, pp 1–54. https://doi.org/10.1007/10_2015_328

Ihnken S, Eggert A, Beardall J (2010) Exposure times in rapid light curves affect photosynthetic parameters in algae. Aquatic Bot 93:185–194. https://doi.org/10.1016/j.aquabot.2010.07.002

Jin X et al (2020) High-Throughput Estimation of Crop Traits: a Review of Ground and Aerial Phenotyping Platforms. IEEE Geoscience and Remote Sensing Magazine. https://doi.org/10.1109/mgrs.2020.2998816

Juergens MT et al (2015) The regulation of photosynthetic structure and function during nitrogen deprivation in *Chlamydomonas reinhardtii*. Plant Physiol 167:558–573. https://doi.org/10.1104/pp.114.250530

Kalaji H et al (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. Photosynth Res 122:121–158. https://doi.org/10.1007/s11120-014-0024-6

Kalaji HM et al (2017) Frequently asked questions about chlorophyll fluorescence the sequel. Photosynth Res 132:13–66. https://doi.org/10.1007/s11120-016-0318-y

Kanwugu ON, Glukhareva TV, Danilova IG, Kovaleva EG (2021) Natural antioxidants in diabetes treatment and management: prospects of astaxanthin. Crit Rev Food Sci Nutr 1-24. https://doi.org/10.1080/10408398.2021.1881434

Kramer DM, Johnson G, Kiirats O, Edwards GE (2004) New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. Photosynth Res 79:209–218. https://doi.org/10.1023/B:PRES.0000015391.99477.0d

Kugler A et al (2019) Long-chain polyunsaturated fatty acids in the green microalga *Lobosphaera incisa* contribute to tolerance to abiotic stresses. Plant Cell Physiol 60:1205–1223. https://doi.org/10.1093/pcp/pcz013

Mallick N (2006) Immobilization of microalgae. In Methods in biotechnology 22:373–391. https://doi.org/10.1007/978-1-59745-053-9_12

Martínez-Roldán AJ, Perales-Vela HV, Cañizares-Villanueva RO, Torzillo G (2013) Physiological response of *Nannochloropsis* sp. to saline stress in laboratory batch cultures. J Appl Phycol 26:115–121. https://doi.org/10.1007/s10811-013-0060-1

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 14:217–232. https://doi.org/10.1016/j.rser.2009.07.020

Maxwell K, Johnson G (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51:659–668. https://doi.org/10.1093/jxb/51.345.659

Minhas AK, Hodgson P, Barrow CJ, Adholeya A (2016) A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. Front Microbiol 7:546. https://doi.org/10.3389/fmicb.2016.00546

Minyuk G, Dantsyuk N, Chelebieva E, Chubchikova I, Drobetskaya I, Solovchenko A (2019) The effect of diverse nitrogen sources in the nutrient medium on the growth of the green microalgae *Chromochloris zofingiensis* in the batch culture. Marine Biological Journal 4:41–52. https://doi.org/10.21072/mbj.2019.04.4.04

Minyuk GS et al (2016) pH and CO_2 effects on *Coelastrella* (*Scotiellopsis*) *rubescens* growth and metabolism. Russ J Plant Physiol 63:566–574. https://doi.org/10.1134/S1021443716040105

Mohana S, Acharya BK, Madamwar D (2009) Distillery spent wash: treatment technologies and potential applications. J Hazard Material 163:12–25. https://doi.org/10.1016/j.jhazmat.2008.06.079

Muñoz R, Guieysse B (2006) Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Res 40:2799–2815. https://doi.org/10.1016/j.watres.2006.06.011

Perri K, Manning S, Watson S, Fowler N, Boyer G (2021) Dark adaptation and ability of pulse-amplitude modulated (PAM) fluorometry to identify nutrient limitation in the bloom-forming cyanobacterium, *Microcystis aeruginosa* (Kützing). J Photochem Photobiol b: Biol 219:112186. https://doi.org/10.1016/j.jphotobiol.2021.112186

Plyusnina TY et al (2020) Gradual changes in the photosynthetic apparatus triggered by nitrogen depletion during microalgae cultivation in photobioreactor. Photosynthetica 58:443–451. https://doi.org/10.32615/ps.2020.002

Porcar-Castell A et al (2021) Chlorophyll *a* fluorescence illuminates a path connecting plant molecular biology to Earth-system science. Nat Plants 7:998–1009. https://doi.org/10.1038/s41477-021-00980-4

Ptushenko VV et al (2021) The effect of chilling on the photosynthetic apparatus of microalga *Lobosphaera incisa* IPPAS C-2047. Biochem Mosc 86:1590–1598. https://doi.org/10.1134/S0006297921120087

Ptushenko VV, Solovchenko AE (2016) Tolerance of the photosynthetic apparatus to acidification of the growth medium as a possible determinant of CO_2-tolerance of the symbiotic microalga *Desmodesmus* sp. IPPAS-2014. Biochem Mosc 81:1531–1537. https://doi.org/10.1134/S0006297916120142

Skjånes K, Rebours C, Lindblad P (2012) Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. Crit Rev Biotechnol 33:172–215. https://doi.org/10.3109/07388551.2012.681625

Solovchenko A, Chivkunova O, Sun WJ, Merzlyak M (2012) Reconstruction of microalgal suspension absorption spectra from reflectance spectra of the cells deposited on GF/F filters. Israel J Plant Sci 60:231–242. https://doi.org/10.1560/IJPS.60.1-2.231

Solovchenko A et al (2015) A novel CO_2-tolerant symbiotic *Desmodesmus* (Chlorophyceae, Desmodesmaceae): acclimation to and performance at a high carbon dioxide level. Algal Res 11:399–410. https://doi.org/10.1016/j.algal.2015.04.011

Solovchenko A, Khozin-Goldberg I (2013) High-CO_2 tolerance in microalgae: possible mechanisms and implications for biotechnology and bioremediation. Biotechnol Lett 35:1745–1752. https://doi.org/10.1007/s10529-013-1274-7

Solovchenko A, Khozin-Goldberg I, Recht L, Boussiba S (2011) Stress-induced changes in optical properties, pigment and fatty acid content of *Nannochloropsis* sp.: implications for non-destructive assay of total fatty acids. Marine Biotechnol 13:527–535. https://doi.org/10.1007/s10126-010-9323-x

Solovchenko A, Khozin I, Chivkunova O (2011) Real-time spectral techniques for detection of the buildup of valuable compounds and stress in microalgal cultures: implications for biotechnology. In: Johansen M (ed) Microalgae: biotechnology, microbiology and energy. Nova Science Publishers, Hauppauge, NY, pp 251–276

Solovchenko A, Lukyanov A, Solovchenko O, Didi-Cohen S, Boussiba S, Khozin-Goldberg I (2014) Interactive effects of salinity, high light, and nitrogen starvation on fatty acid and carotenoid profiles in *Nannochloropsis oceanica* CCALA 804. Eur J Lipid Sci Technol 116:635–644. https://doi.org/10.1002/ejlt.201300456

Solovchenko A, Neverov K (2017) Carotenogenic response in photosynthetic organisms: a colorful story. Photosynth Res 133:31–47. https://doi.org/10.1007/s11120-017-0358-y

Solovchenko A et al (2014) Phycoremediation of alcohol distillery wastewater with a novel Chlorella sorokiniana strain cultivated in a photobioreactor monitored on-line via chlorophyll fluorescence. Algal Res 6:234–241. https://doi.org/10.1016/j.algal.2014.01.002

Solovchenko A, Solovchenko O, Khozin-Goldberg I, Didi-Cohen S, Pal D, Cohen Z, Boussiba S (2013) Probing the effects of high-light stress on pigment and lipid metabolism in nitrogen-starving microalgae by measuring chlorophyll fluorescence transients: studies with a Δ5 desaturase mutant of Parietochloris incisa (Chlorophyta Trebouxiophyceae). Algal Res 2:175–182. https://doi.org/10.1016/j.algal.2013.01.010

Solovchenko A (2013) Physiology and adaptive significance of secondary carotenogenesis in green microalgae. Russ J Plant Physiol 60:1–13. https://doi.org/10.1134/S1021443713010081

Solovchenko A, Vasilieva S, Zaitsev P, Lukyanov A, Skripnikova E, Antal T (2022) Approaches to rapid screening of pharmaceutical xenobiotic effects on microalgae via monitoring of photosynthetic apparatus condition. J Appl Phycol 34:353–361. https://doi.org/10.1007/s10811-021-02660-4

Stirbet A, Riznichenko GY, Rubin AB, Govindjee, (2014) Modeling chlorophyll *a* fluorescence transient: relation to photosynthesis. Biochem Mosc 79:291–323. https://doi.org/10.1134/S0006297914040014

Strasser R, Tsimilli-Michael M, Srivastava A (2004) Analysis of the chlorophyll *a* fluorescence transient. In: Papageorgiou G, Govindjee (eds) Chlorophyll *a* fluorescence: a signature of photosynthesis. Springer, 321–362. https://doi.org/10.1007/978-1-4020-3218-9_12

Tian L, Nawrocki WJ, Liu X, Polukhina I, van Stokkum IHM, Croce R (2019) pH dependence, kinetics and light-harvesting regulation of nonphotochemical quenching in *Chlamydomonas*. Proc Natl Acad Sci USA 116:8320–8325. https://doi.org/10.1073/pnas.1817796116

Tsygankov A, Kosourov S (2014) Immobilization of photosynthetic microorganisms for efficient hydrogen production. In: Zannoni D, De Philippis R (eds) Microbial bioenergy: hydrogen production. Advances in Photosynthesis and Respiration. Springer Science+Business Media, Dordrecht, 38, 321–347. https://doi.org/10.1007/978-94-017-8554-9_14

Van Den Hende S, Vervaeren H, Boon N (2012) Flue gas compounds and microalgae: biochemical interactions leading to biotechnological opportunities. Biotechnol Adv 30:1405–1424. https://doi.org/10.1016/j.biotechadv.2012.02.015

Vasilieva S et al (2021) Bio-inspired materials for nutrient biocapture from wastewater: microalgal cells immobilized on chitosan-based carriers. J Water Proc Eng 40:101774. https://doi.org/10.1016/j.jwpe.2020.101774

Vítová M, Bišová K, Kawano S, Zachleder V (2015) Accumulation of energy reserves in algae: from cell cycles to biotechnological applications. Biotechnol Adv 33:1204–1218. https://doi.org/10.1016/j.biotechadv.2015.04.012

Vonshak A, Novoplansky N, Silva Benavides AM, Torzillo G, Beardall J, Palacios YM (2020) Photosynthetic characterization of two *Nannochloropsis* species and its relevance to outdoor cultivation. J Appl Phycol 32:909–922. https://doi.org/10.1007/s10811-019-01985-5

Zorin B et al (2017) Arachidonic acid is important for efficient use of light by the microalga *Lobosphaera incisa* under chilling stress. Biochim Biophys Acta (BBA)–Molecular and Cell Biology of Lipids 1862:853–868. https://doi.org/10.1016/j.bbalip.2017.04.008