ISSN 0096-3925, Moscow University Biological Sciences Bulletin, 2023, Vol. 78, No. 3, pp. 156–162. © Allerton Press, Inc., 2023.
Russian Text © The Author(s), 2023, published in Vestnik Moskovskogo Universiteta, Seriya 16: Biologiya, 2023, Vol. 78, No. 3, pp. 170–177.

RESEARCH ARTICLE

Evaluation of the Primary Photosynthesis Reactions in Microalgae Single Cell by the Microfluorimetric Method

A. A. Volgusheva[a] (ORCID: 0000-0002-0414-1236), I. V. Konyukhov[a] (ORCID: 0000-0002-7686-461X), and T. K. Antal[b,*] (ORCID: 0000-0002-9690-8034)

[a] *Department of Biophysics, Faculty of Biology, Moscow State University, Moscow, 119234 Russia*
[b] *Laboratory of Integrated Environmental Research, Pskov State University, Pskov, 180000 Russia*
**e-mail: taras_an@mail.ru*

Received May 8, 2023; revised September 12, 2023; accepted September 20, 2023

Abstract—High-resolution chlorophyll fluorescence light induction curves (OJIP transients) are widely used to assess the primary photosynthetic responses of phototrophic cells. Chlorophyll fluorescence measuring methods coupled with microscopy techniques provide a promising opportunity to measure OJIP transients on individual algae cells, allowing scientists to investigate stress adaptation mechanisms related to reorganization of the microalgae population or the phytoplankton community. In this work, the authors first characterized the OJIP transients measured on individual algae cells using the original microfluorimeter and compared them with OJIP transients recorded in microalgae suspensions. Based on the results of the study, a method is proposed for analyzing OJIP curves of individual microalgae cells and ways to further improve microfluorimeters.

Keywords: photosynthesis, OJIP transient, JIP-test, chlorophyll fluorescence, microfluorimetry, microalgae
DOI: 10.3103/S0096392523700050

INTRODUCTION

Photosynthetic activity is one of the key indicators of the state of phototrophic cells, which is widely used in studying the cell responses to the influence of unfavorable environmental factors. Typically, methods are used to measure the rate of oxygen photoproduction and/or carbon dioxide uptake to assess the photosynthetic activity of laboratory cultures of microalgae or phytoplankton communities [1–3]. These methods are quite labor-intensive, especially in field conditions, and require additional determination of biomass or chlorophyll content (Chl). Therefore, as an alternative to direct methods, optical methods for measuring the fast chlorophyll fluorescence (CF) signal have become widespread [4, 5]. This approach is based on the ability of Chl to emit light quanta (fluorescence) during illumination. In this case, the yield of CF is associated with the efficiency of using light energy in the primary reactions of photosynthesis associated with the reduction of $NADP^+$ and the synthesis of ATP to supply energy to the Calvin cycle.

Pulse amplitude modulation (PAM) and methods for recording kinetic curves of light induction and dark relaxation of high-resolution fast CF are presently among the most popular methods for measuring CF [6–8]. Among the latter, the most common method for recording the CF light induction curve is the so-called OJIP curve, which mainly reflects the process of reduction of the photosynthetic electron transport chain (ETC) under high intensity light [9]. When recording an OJIP curve, photosynthetic photon flux density (PPFD) of photosynthetically active radiation usually does not exceed 10 000 μmol photons m^{-2} s^{-1}.

A typical OJIP curve reflects the increase in CF yield from the minimum value F_O (point O on the curve) in the dark-adapted state, when the photosynthetic ETC is in an oxidized state, to the maximum level F_M (peak P), when electron carriers acquire a completely reduced state. The presence of three phases of fluorescence yield growth—OJ (from ~5–40 to 2–3 ms), JI (from 2–3 to 20–30 ms), and IP (from 20–30 to 100–300 ms)—reflects three consecutive process of induction of photosynthetic electron transport. The OJ phase (the so-called photochemical phase) depends on the intensity of the exciting light and is characterized by the reduction of the acceptor side of photosystem 2 (PSII): quinone acceptors Q_A and Q_B [10]. It is believed that the increase in CF yield subsequent to the J point is due to additional reduction of the acceptor side of PSII, the plastoquinone pool (PQ pool), an increase in the energization of thylakoid membranes, and conformational changes of PSII [8, 11, 12].

Recently, methods for measuring CF have become widespread, making it possible to assess the state of individual cells of phototrophic cells. This makes it

possible to actively study the mechanisms of adaptation of the microalgae population to the effects of stress factors due to the reorganization of the population structure. This adaptation mechanism is based on the high genetic variability of single-celled organisms, which allows them to survive even in extreme conditions. Previously, it was proposed to use the PAM method in combination with a microscope to assess the photosynthetic activity of individual algal cells [13]. Methods for recording OJIP curves on individual cells have been developed to a much lesser extent.

Recently, a microfluorimeter has been developed at the Department of Biophysics, Faculty of Biology, Moscow State University; it makes it possible to record OJIP curves on individual microalgae cells [14]. The analysis of OJIP curves proposed by the authors is based on determining the value of the maximum variable CF (F_V) and the basic indicators of the primary reactions of photosynthesis: the maximum quantum yield of the photochemical conversion of light energy into PSII (F_V/F_M) and the efficiency of the photochemical conversion of light energy in PSII under actinic light ($F_{V'}/F_{M'}$). These indicators make it possible to determine the share of PSII centers in the cell capable of photochemical energy conversion. Using the developed method, cells of the green microalgae *Chlorella sorokiniana* were identified, which retain high photochemical activity and viability in the presence of a strong toxicant that causes death of up to 98% of cells [15].

The multiphase OJIP curve allows one to determine other important parameters of photosynthetic electron transport (in particular, the efficiency of electron transfer from PSII to the PQ pool) in addition to the parameters of the photochemical activity of PSII [16]. In this work, we characterized OJIP curves obtained on individual cells of green and diatom microalgae for the first time and proposed to estimate the quantum yield of electron transport in PSII for a more complete analysis of the primary reactions of photosynthesis.

MATERIALS AND METHODS

Cultivation of microalgae. Green algae (*Scenedesmus quadricauda, Chlamydomonas reinhardtii)* and diatoms (*Gomphonema parvulum, Sellaphora* sp.) were used. Diatoms and *S. quadricauda* were grown on BG11 medium [17]. When growing diatoms, $Na_2SiO_3 \cdot 9H_2O$ at a concentration of 0.030 g L^{-1} and vitamins B1 and B12 (5×10^{-7} g L^{-1}) were added to the standard BG11 medium. *C. reinhardtii* were grown on HS mineral medium [18]. Microalgae cultures were grown in 100-mL Erlenmeyer flasks on a shaker with a stirring rate of 120 rpm. min^{-1} and a temperature of 25°C. The illumination regime regime was 10 h of light (PPFD 100 μmol photons m^{-2} s^{-1}) and 14 h of darkness. The cultures were grown for 3 days, and the final Chl concentration was ~3 μg mL^{-1}.

Cultivation of C. reinhardtii on a sulfur-free medium. In order to create conditions for cell sulfur starvation, *C. reinhardtii* culture was grown on complete HS medium, and then was placed on sulfur-free HS medium, in which sulfate was replaced by an equimolar amount of chloride. The protocol for transferring cells to sulfur-free medium consisted of sedimentation of the culture by centrifugation (3000*g*, 5 min) and subsequent resuspension in sulfur-free HS medium. This procedure was repeated three times. Control cells were pelleted and resuspended in complete HS medium three times. After this, 10 mL of culture with a Chl concentration of 3–4 μg mL^{-1} was placed in 100-mL Erlenmeyer flasks and incubated under the same conditions that were used when growing other cell cultures.

Registration and analysis of OJIP curves. OJIP curves in the microalgae suspensions were recorded using a Portable Fluorimeter Spectral and Fluorescence Kinetic System (PSFKS) [15], developed at the Department of Biophysics, Faculty of Biology, Moscow State University (Moscow, Russia) and in the Laboratory of Integrated Environmental Research at Pskov State University (Pskov, Russia). The excitation source for CF was blue light with a maximum at 445 nm and a PPFD of 7500 μmol photons m^{-2} s^{-1}. Before measurements, cells were kept in the dark for 3 min. The induction curve was recorded for each sample with ten technical replicates and subsequent averaging.

Registration of OJIP curves on individual microalgae cells was carried out using a microfluorimeter developed at the Department of Biophysics, Faculty of Biology, Moscow State University, as described previously [14, 15]. Before measurements, 0.3% alcohol was added to the microalgae suspension culture to immobilize the cells; they were adapted to the dark for 3 min and then placed into the Goryaev counting chamber. Ten induction curves were recorded per each cell. We used the parameters of the JIP test [16] to analyze the OJIP curves (Table 1).

RESULTS

Normalized OJIP curves obtained in suspensions and on individual cells of green microalgae *S. quadricauda* (Fig. 1a), *C. reinhardtii* (Fig. 1b), and diatoms *G. parvulum* (Fig. 1c), and *Sellaphora* sp. (Fig. 1d) are presented. The shape of the curves in suspensions and on cells generally corresponds to the OJIP curve typical for microalgae, revealing the main stages of growth in CF yield. The curves were normalized for easier comparison to the value of the maximum variable CF (F_V). The main difference of the curves obtained on individual cells as compared to those in cell suspensions was a faster initial increase in CF yield (OJ phase). This was achieved due to the absence of a lag

Table 1. List of fluorescence parameters and equations used in the JIP test and explanations for them [16]

Fluorescence parameters measured	
F_O	CF intensity at 50 μs
F_J	CF intensity at 2 ms
F_I	CF intensity at 20 ms
F_P (=F_M)	CF output
JIP test parameters	
$F_V = F_M - F_O$	Maximum variable CF
$V_J = (F_J - F_O)/F_V$	Relative amplitude of O–J phase
$V_I = (F_I - F_J)/F_V$	Relative amplitude of J–I phase
$TR_O/ABS = F_V/F_M = (F_M - F_O)/F_M$	Maximum quantum yield of photochemical energy conversion in PSII
$1–Vj = 1 – ((F_J - F_O)/F_V)$	Efficiency of electron transfer from PSII to PQ pool
$ETo/ABS = (F_V/F_M) \times (1–Vj)$	Maximum quantum yield of electron transport in PSII

phase preceding the rapid increase in CF. Moreover, the inflection point I and, accordingly, the IP phase did not visually appear on the OJIP curves of individual diatom cells. It should be noted that the IP phase was also suppressed in the OJIP curves of individual green algae cells.

The data calculated using the OJIP curves shown in Fig. 1 were used to obtain the values of F_V/F_M, (1–Vj), and ETo/ABS (Table 2). The results obtained on individual cells of four microalgae cultures were characterized by underestimated F_V/F_M values (by 10–15%) compared to these indicators for suspensions. At the

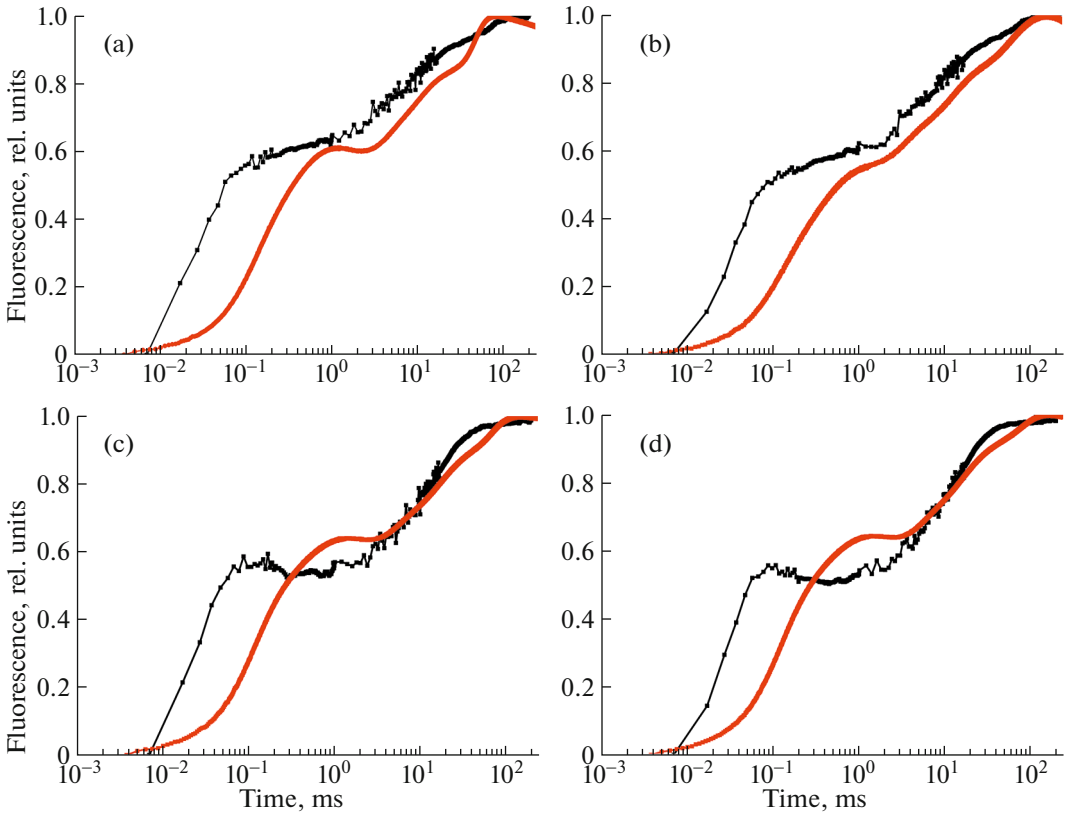

Fig. 1. OJIP curves measured in cell suspensions (red) and individual cells (black) of green microalgae (a) *S. quadricauda* and (b) *C. reinhardtii*; and diatoms (c) *G. parvulum* and (d) *Sellaphora* sp. The curves are normalized to the maximum value of the CF variable (OJIP amplitude).

Table 2. JIP test parameters calculated based on fluorescence data obtained from OJIP curves (see Fig. 1)

	Individual cells			Cell suspensions		
Culture	**F_V/F_M**	**1–Vj**	**ETo/ABS**	**F_V/F_M**	**1–Vj**	**ETo/ABS**
S. quadricauda	0.66 ± 0.08	0.37 ± 0.10	0.25 ± 0.08	0.73 ± 0.05	0.37 ± 0.02	0.29 ± 0.02
C. reinhardtii	0.65 ± 0.06	0.43 ± 0.05	0.28 ± 0.05	0.77 ± 0.01	0.43 ± 0.01	0.33 ± 0.01
C. reinhardtii (-S)*	0.51 ± 0.05	0.37 ± 0.04	0.19 ± 0.09	0.64 ± 0.02	0.36 ± 0.01	0.23 ± 0.01
G. parvulum	0.65 ± 0.09	0.43 ± 0.06	0.27 ± 0.04	0.76 ± 0.01	0.34 ± 0.01	0.26 ± 0.01
Sellaphora sp.	0.67 ± 0.07	0.44 ± 0.06	0.29 ± 0.04	0.76 ± 0.01	0.37 ± 0.03	0.27 ± 0.03

(-S) *C. reinhardtii* culture was incubated for 24 h on sulfur-free medium.

same time, the value of the 1–Vj parameter was approximately 20% higher in individual diatom cells compared to suspensions. The ETo/ABS in individual cells of green microalgae was 0.25 (*S. quadricauda*) and 0.29 (*C. reinhardtii*) and was 0.29 (*S. quadricauda*) and 0.33 (*C. reinhardtii*) in suspensions. In individual cells of diatoms, ETo/ABS was 0.27 (*G. parvulum*) and 0.29 *(Sellaphora* sp.). Similar values were obtained for suspensions: 0.26 (*G. parvulum*) and 0.27 *(Sellaphora* sp.).

The effect of 24-h incubation of the *C. reinhardtii* culture on sulfur-free medium on CF parameters was also estimated (Table 2). Stress exposure induced a decrease in the CF parameters F_V/F_M and 1–Vj by 17 and 16%, respectively, when measured in suspensions, and by 22 and 15%, respectively, when measured on individual cells. The quantum yield of electron transport in PSII (ETo/ABS) was reduced by 32 and 30% when measured in cell suspensions and on individual cells, respectively.

Registration of OJIP curves in a representative sample of individual cells (at least 100 cells per each culture) made it possible to obtain the distribution of F_V/F_M and 1–Vj values in the populations of the control and sulfur-starved *C. reinhardtii* cultures (Fig. 2). In the control, approximately 70% of the cells were characterized by high F_V/F_M ranging from 0.6 to 0.7, the remaining cells had values in the range of 0.5–0.6. The distribution of 1–Vj in the control culture was similar: most of the cells (approximately 75%) were characterized by high values (0.4–0.5), while the remaining cells showed 0.3–0.4. Sulfur deficiency in the medium induced the appearance of additional cell fractions with low values of these parameters. Thus, the size of the cell fraction with high F_V/F_M values (0.6–0.7) decreased from 70% in the control to 20% in sulfur-depleted cells, and the one with F_V/F_M values of 0.5–0.6 increased from 30 to 36%. The sizes of new cell fractions with low F_V/F_M values were 34% (range of 0.4–0.5) and 10% (0.3–0.4). The effect of sulfur deficiency on the distribution of the 1–Vj parameter was less pronounced compared to its effect on F_V/F_M. Thus, approximately 60% of starving cells maintained 1–Vj values > 0.4, 33% of cells had values from 0.3 to 0.4, and 10% of cells showed 0.2–0.3.

DISCUSSION

To date, there is no description in the literature of OJIP curves obtained from individual algal cells. Our comparison of OJIP curves in individual cells and in cell suspensions of green and diatom microalgae revealed a number of features in the shape of the curves in individual cells, which were determined primarily by differences in the intensity of the exciting

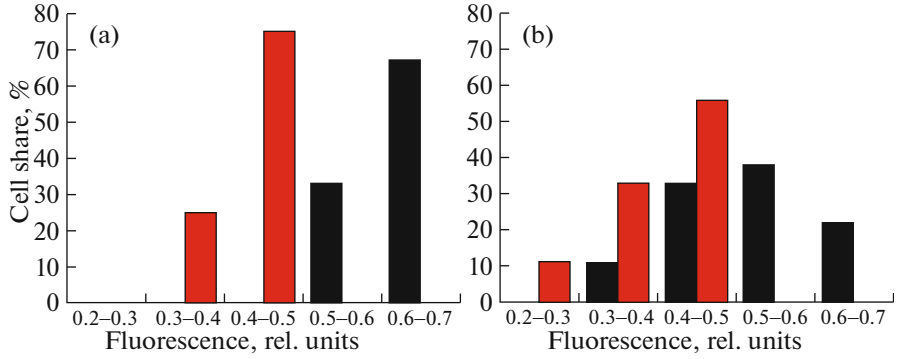

Fig. 2. Distribution of CF parameters F_V/F_M (black) and 1–Vj (red) in the control culture of (a) *C. reinhardtii* and after 24 h of incubation (b) without sulfur. Parameters are calculated based on fluorescence data obtained from OJIP curves recorded on individual cells using a microfluorimeter. The sample for each culture was at least 100 cells.

light in the two fluorimeters used. Thus, measurements in suspensions were carried out at the maximum possible light intensity for the PSFKS device (PPFD 7500 μmol photons $m^{-2} s^{-1}$), while PPFD was twice as high (15000 μmol photons $m^{-2} s^{-1}$) in the microfluorimeter. Such high light intensity is necessary to increase the overall CF yield and to consequently reduce the noise resulting from the low signal from an individual cell.

The high intensity of the exciting light in the microfluorometer was the most likely reason for the underestimation of the yield of the CF variable when measuring on individual cells and for the underestimation of the F_V/F_M by 10–15% compared to the results of measurements in cell suspensions (Table 2). This effect may be due to the rapid development of nonphotochemical quenching as a result of high energization of thylakoid membranes, leading to a decrease in CF yield, especially during the last IP phase. Indeed, the IP phase was suppressed in the OJIP curves of individual cells of green microalgae, and it did not appear at all in diatom microalgae (Fig. 1). The possibility that nonphotochemical quenching may be the cause of IP phase suppression is supported by the fact that some diatom microalgae with strong nonphotochemical quenching have virtually no IP phase when measured in cell suspensions. However, it appears after treatment of the culture with the ionophore valinomycin [19].

It is known that the rate of CF growth during the OJ phase depends on the intensity of the exciting light [9]. When recording OJIP curves on individual cells, a rapid increase in CF began only 7–8 μs after the start of illumination, and the OJ phase had an exponential shape, while a rapid increase in CF was observed 40–50 μs after the start of illumination when measured in suspensions. In this case, the OJ phase was characterized by a sigmoid shape (Fig. 1). This observation confirms the assumption of a significant influence of the excitation light intensity on the shape of OJIP curves in individual cells.

In hydrobiological and environmental studies, fluorescence methods are usually used to determine the basic characteristic of photosynthesis, i.e., the maximum quantum yield of photochemical energy conversion to PSII (F_V/F_M) [4, 5, 20, 21]. This characteristic reflects the very first stage of conversion of the exciton energy in the PSII antenna into the energy of separated charges $P^+_{680} Q^-_A$. The next most important step in photosynthesis is the transfer of an electron from PSII to the PQ pool. This process depends on the functional state of the oxygen-evolving complex and the acceptor side of PSII, which is influenced by stress factors [22, 23]. It should be noted that inhibition of the acceptor side of PSII (for example, by diuron) leads to a complete blocking of photosynthetic electron transport and photosynthesis in general, while the F_V/F_M remains practically unchanged. In this regard, situations are possible wherein the observed values of F_V/F_M in phytoplankton do not reveal inhibition of photosynthesis: in particular, when natural water bodies are polluted with herbicides.

Previously, Strasser proposed a method for analyzing OJIP curves (JIP test) based on a model assuming that the shape of the curve reflects the three-stage process of reducing Q_A in PSII [16]. Currently, the proposed model is very popular, although it is subject to fair criticism since it does not take into account the influence of the electrochemical potential on the thylakoid membrane and conformational changes in PSII on the CF yield [11, 12]. Nevertheless, the key parameters of the JIP test are empirically confirmed. One of these parameters is 1–Vj, which reflects the efficiency of electron transfer from Q_A to the PQ pool. This parameter is characterized by high sensitivity to influences affecting electron transport on the donor and acceptor sides of PSII [16]. Parameters 1–Vj and F_V/F_M allow us to determine the quantum yield of electron transport in PSII (ETo/ABS), which characterizes the conversion of an exciton in the PSII antenna into an electron transferred to the PQ pool. ETo/ABS values ranged from 0.25 to 0.29 when measured in individual cells and from 0.26 to 0.33 when measured in suspensions (Table 2), indicating some differences in the efficiency of primary photosynthetic reactions among different microalgae cultures and a slight underestimation of this parameter when measured on individual cells.

The mechanisms of adaptation of microalgae to stress include multiple processes of reorganization of metabolic processes, cell ultrastructure, and population structure [24, 25]. Usually, to study adaptation processes, physiological and other cell characteristics averaged over a culture are assessed, without taking into account their variations in the population. However, the heterogeneity of the population in terms of physiological characteristics can ensure its recovery after a decrease in stress pressure. Analysis of OJIP curves of individual microalgae cells can be used to study the distribution of key indicators of photosynthetic activity in a microalgae population under stress conditions [15].

Nutrient deficiency deficiency is the most pressing stress for natural phytoplankton. The effect of sulfur deficiency on the functional state of the photosynthetic apparatus and OJIP curves has been well studied in connection with the ability of some microalgae to produce hydrogen [26, 27]. This stress induces multiple changes in the photosynthetic apparatus: in particular, a decrease in the number of photochemically active PSII centers, disruption of linear electron transport in the chloroplast, and rapid inactivation of the Calvin cycle. In this work, we studied the effect of sulfur deficiency on the distribution of F_V/F_M and 1–Vj in the *C. reinhardtii* culture. Stressful conditions led to an increase in population heterogeneity in these parameters, reflecting the division of the population

into fractions of tolerant and stress-sensitive cells. The parameters F_V/F_M and 1–Vj were characterized by a similar distribution in the control, but differences appeared under conditions of sulfur deficiency (Fig. 2). Thus, the parameter 1–Vj was characterized by higher stability under stress conditions compared to F_V/F_M since a significant proportion remained in the population cells with high values of 1–Vj similar to control. This result indicates that the decrease in the activity of primary photosynthetic reactions under conditions of sulfur deficiency occurred mainly as a result of a decrease in the content of photochemically active PSII centers in the cell, while the decrease in the efficiency of electron transport on the acceptor side of photochemically active PSII centers was insignificant.

CONCLUSIONS

Measuring the OJIP curves of individual phytoplankton cells opens up the prospect of studying the mechanisms of reorganization of the community structure of phototrophic cells to ensure survival under stress conditions. To analyze OJIP curves recorded on individual cells, we propose to use empirically verified CF parameters (F_V/F_M and 1–Vj), which allow us to estimate the quantum yield of electron transport in PSII. Further improvement of the technical qualities of the microfluorimeter presented in the work, primarily by increasing the sensitivity of the CF detector and reducing the intensity of the exciting light, will make it possible to more accurately assess the activity of the primary reactions of photosynthesis.

FUNDING

The research was funded by the Russian Science Foundation, project no. 23-24-00353.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Bender, M., Grande, K., Johnson, K., Marra, J., Williams, P.J., Sieburth, J., Pilson, M., Langdon, C., Hitchcock, G., Orchardo, J., Hunt, C., Donaghay, P., and Heinemann, K., A comparison of four methods for determining planktonic community production1, *Limnology Oceanography*, 1987, vol. 32, no. 5, pp. 1085–1098. https://doi.org/10.4319/lo.1987.32.5.1085
2. Kelly, C.A., Fee, E., Ramlal, P.S., Rudd, J.W.M., Hesslein, R.H., Anema, C., and Schindler, E.U., Natural variability of carbon dioxide and net epilimnetic production in the surface waters of boreal lakes of different sizes, *Limnology Oceanography*, 2001, vol. 46, no. 5, pp. 1054–1064. https://doi.org/10.4319/lo.2001.46.5.1054
3. Del Giorgio, P.A. and Williams, P.J., Respiration in aquatic ecosystems: History and background, *Respiration in Aquatic Ecosystems*, Del Giorgio, P.A. and Williams, P.J., Eds., New York: Oxford Univ. Press, vol. 2023, pp. 1–17.
4. Matorin, D.N. and Goryachev, S.N., *Fluorestsentsiya khlorofilla mikrovodoroslei v biotestirovanii zagryaznenii* (Fluorescence of Algae Chlorophyll in Biotesting of Contaminations), Moscow: Al'teks, 2017.
5. Pogosyan, S.I., Konyukhov, I.V., and Rubin, A.B., *Problemy ekologicheskoi biofiziki* (Problems of Ecological Biophysics), Moscow–Izhevsk: Izhevskii Inst. Komp'yuternykh Issled., 2017.
6. Schreiber, U., Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: An overview, *Chlorophyll a Fluorescence*, Papageorgiou, G.C. and Govindjee, Eds., Advances in Photosynthesis and Respiration, Berlin: Springer, 2004, vol. 19, pp. 279–319. https://doi.org/10.1007/978-1-4020-3218-9_11
7. Papageorgiou, G.C., Tsimilli-Michael, M., and Stamatakis, K., The fast and slow kinetics of chlorophyll a fluorescence induction in plants, algae and cyanobacteria: A viewpoint, *Photosynthesis Res.*, 2007, vol. 94, nos. 2–3, pp. 275–290. https://doi.org/10.1007/s11120-007-9193-x
8. Stirbet, A. and Govindjee, Chlorophyll a fluorescence induction: A personal perspective of the thermal phase, the J–I–P rise, *Photosynthesis Res.*, 2012, vol. 113, nos. 1–3, pp. 15–61. https://doi.org/10.1007/s11120-012-9754-5
9. Lazár, D., The polyphasic chlorophyll a fluorescence rise measured under high intensity of exciting light, *Funct. Plant Biol.*, 2006, vol. 33, no. 1, pp. 9–30. https://doi.org/10.1071/fp05095
10. Schansker, G., Tóth, S.Z., Kovács, L., Holzwarth, A.R., and Garab, G., Evidence for a fluorescence yield change driven by a light-induced conformational change within photosystem II during the fast chlorophyll a fluorescence rise, *Biochim. Biophys. Acta (BBA) Bioenerg.*, 2011, vol. 1807, no. 9, pp. 1032–1043. https://doi.org/10.1016/j.bbabio.2011.05.022
11. Vredenberg, W.J. and Bulychev, A., Photoelectric effects on chlorophyll fluorescence of photosystem II in vivo. Kinetics in the absence and presence of valinomycin, *Bioelectrochemistry*, 2003, vol. 60, nos. 1–2, pp. 87–95. https://doi.org/10.1016/s1567-5394(03)00053-7
12. Sipka, G., Magyar, M., Mezzetti, A., Akhtar, P., Zhu, Q., Xiao, Ya., Han, G., Santabarbara, S., Shen, J.-R., Lambrev, P.H., and Garab, G., Light-adapted charge-separated state of photosystem II: Structural and functional dynamics of the closed reaction center, *Plant Cell*, 2021, vol. 33, no. 4, pp. 1286–1302. https://doi.org/10.1093/plcell/koab008
13. Murchie, E.H. and Lawson, T., Chlorophyll fluorescence analysis: A guide to good practice and under-

standing some new applications, *J. Exp. Bot.*, 2013, vol. 64, no. 13, pp. 3983–3998. https://doi.org/10.1093/jxb/ert208

14. Kuznetsov, A.G., Konyukhov, I.V., Pogosyan, S.I., and Rubin, A.B., Microfluorimeter for studying the state of photosynthetic apparatus of individual cells of microalgae, *Oceanology*, 2021, vol. 61, no. 6, pp. 1055–1063. https://doi.org/10.1134/s0001437021060278
15. Volgusheva, A.A., Todorenko, D.A., Konyukhov, I.V., Voronova, E.N., Pogosyan, S.I., Plyusnina, T.Yu., Khruschev, S.S., and Antal, T.K., Acclimation response of green microalgae *Chlorella sorokiniana* to 2,3',4,4', 6-Pentachlorobiphenyl, *Photochem. Photobiol.*, 2022, vol. 99, no. 4, pp. 1106–1114. https://doi.org/10.1111/php.13771
16. Strasser, R.J., Tsimilli-Michael, M., and Srivastava, A., Analysis of the chlorophyll a fluorescence transient, *Chlorophyll a Fluorescence*, Papageorgiou, G.C. and Govindjee, Eds., Advances in Photosynthesis and Respiration, vol. 19, Dordrecht: Springer, 2004, pp. 321–362. https://doi.org/10.1007/978-1-4020-3218-9_12
17. Rippka, R., Stanier, R.Y., Deruelles, J., Herdman, M., and Waterbury, J.B., Generic assignments, strain histories and properties of pure cultures of cyanobacteria, *Microbiology*, 1979, vol. 111, no. 1, pp. 1–61. https://doi.org/10.1099/00221287-111-1-1
18. Harris, E.H., *The Chlamydomonas Sourcebook: A Comprehensive Guide to Biology and Laboratory Use*, San Diego, Calif.: Academic, 1989.
19. Antal, T.K., Osipov, V., Matorin, D.N., and Rubin, A.B., Membrane potential is involved in regulation of photosynthetic reactions in the marine diatom *Thalassiosira weissflogii, J. Photochem. Photobiol. B: Biol.*, 2011, vol. 102, no. 2, pp. 169–173. https://doi.org/10.1016/j.jphotobiol.2010.11.005
20. Vanharanta, M., Elovaara, S., Franklin, D.J., Spilling, K., and Tamelander, T., Viability of pico- and nanophytoplankton in the Baltic Sea during spring, *Aquat. Ecol.*, 2020, vol. 54, no. 1, pp. 119–135. https://doi.org/10.1007/s10452-019-09730-3
21. Swoczyna, T., Kalaji, H.M., Bussotti, F., Mojski, J., and Pollastrini, M., Environmental stress—What can we learn from chlorophyll a fluorescence analysis in woody plants? A review, *Front. Plant Sci.*, 2022, vol. 13, p. 1048582. https://doi.org/10.3389/fpls.2022.1048582
22. Kalaji, H.M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I.A., Cetner, M.D., Łukasik, I., Goltsev, V., and Ladle, R.J., Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions, *Acta Physiologiae Plant.*, 2016, vol. 38, no. 4, p. 102. https://doi.org/10.1007/s11738-016-2113-y
23. Sasi, S., Venkatesh, J., Daneshi, R., and Gururani, M., Photosystem II extrinsic proteins and their putative role in abiotic stress tolerance in higher plants, *Plants*, 2018, vol. 7, no. 4, p. 100. https://doi.org/10.3390/plants7040100
24. Behrenfeld, M.J. and Milligan, A.J., Photophysiological expressions of iron stress in phytoplankton, *Annu. Rev. Mar. Sci.*, 2013, vol. 5, no. 1, pp. 217–246. https://doi.org/10.1146/annurev-marine-121211-172356
25. Van De Waal, D.B. and Litchman, E., Multiple global change stressor effects on phytoplankton nutrient acquisition in a future ocean, *Philos. Trans. R. Soc. B: Biol. Sci.*, 2020, vol. 375, no. 1798, p. 20190706. https://doi.org/10.1098/rstb.2019.0706
26. Antal, T.K., Krendeleva, T.E., and Tyystjärvi, E., Multiple regulatory mechanisms in the chloroplast of green algae: Relation to hydrogen production, *Photosynthesis Res.*, 2015, vol. 125, no. 3, pp. 357–381. https://doi.org/10.1007/s11120-015-0157-2
27. Petrova, E.V., Kukarskikh, G.P., Krendeleva, T.E., and Antal, T.K., The mechanisms and role of photosynthetic hydrogen production by green microalgae, *Microbiology*, 2020, vol. 89, no. 3, pp. 251–265. https://doi.org/10.1134/s0026261720030169

Translated by D. Martynova