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EXPERIMENTAL ARTICLES

Effect of Chromate Ions on Marine Microalgae *Phaeodactylum tricornutum*

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Abstract—Effect of chromate ions on the culture of a marine diatom *Phaeodactylum tricornutum* was studied using an M-PEA-2 fluorimeter, which carries out simultaneous measurement of fluorescence induction and redox transformations of the P_{700} pigment within a millisecond range. Chromate ions were shown to inhibit electron transport in PS II and decrease the rate of Q_A reduction. This results in decreased values of the quantum yield of electron transport in PS II (φ_{Eo}) and performance index (PI_{ABS}), lower rates of P_{700} reduction, and increased energy (DI_0/RC) and ΔpH-dependent nonphotochemical quenching (q_E). Emergence of the slow component of P_{700} reduction was observed, indicating the activation of cyclic transport in the presence of chromate. Performance index (PI_{ABS}), which was the most sensitive parameter, may be recommended for detection of chromate ions at early stages of their toxic action. The fluorescence parameter F_O is promising application in biotesting to assess the algal growth rates.

Keywords: Phaeodactylum tricornutum, chromate ions, chlorophyll fluorescence, photosynthesis, biotesting, JIP test, M-PEA-2

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Chrome compounds, especially chromate ions, which are present in wastewater of many industries, are toxic for aquatic environments. These compounds are also recommended as a standard toxicant in the standard biotesting of sensitivity of algal cultures (Zayadan and Matorin, 2015).

This toxicant was shown to impair the coordination of metabolic processes in microalgae cells, inhibit their motility, decrease cell number, cause a decrease in chlorophyll concentration, and affect photosynthesis (Rai et al., 2004; Rodríguez et al., 2007; Rocchetta, Küpper, 2009; Giloni-Lima et al., 2010;). Chromate ions were shown to inhibit photosynthesis by affecting the reactions in photosystem II (PS II) (Appenroth et al., 2001; Ali et al., 2006; Hörcsik, 2007; Didur et al., 2013).

Up-to date knowledge on electron transfer in the photosynthetic electron transport chain (ETC) suggested that both photosystem I and photosystem II are involved in stepwise electron transfer. Electron carriers reduced by PSII act as electron donors for PS I. According to the Z-scheme of ETC, activity of each photosystem affects the redox state of the other one. This interaction between PS II and PS I results in chlorophyll fluorescence. The fluorescence value depends on the redox state of the quinone acceptor Q_A. Photoreaction of PS II results in Q_A reduction and increases the fluorescence level, whereas PSI activity leads to Q_A oxidation and decreases the fluorescence level (Lazár and Schansker, 2009; Strasser et al., 2010). Kinetics of light induction of variable fluorescence within a millisecond range reflects the kinetic of electron transfer in PS II and between PS II and PS I (Lazár and Schansker, 2009; Strasser et al., 2010; Goltsev et al., 2014). Measurements of fluorescence induction are widely used in microbiological and ecological studies. Measurement of absorption changes at 820-830 nm (redox state of chlorophyll P_{700} of photosystem I) makes it possible to monitor the state of PS I. An M-PEA-2 fluorimeter makes it possible to register both chlorophyll fluorescence and the redox state of P_{700}, i.e. to monitor separate reactions of PS II and PS I and to register induced changes of delayed fluorescence (DF) (Susplugas et al., 2000; Matorin et al., 2012; Goltsev et al., 2014). However, this approach has not been thus far used for marine algae.

In the present study, changes of photosynthetic processes in marine alga *Phaeodactylum tricornutum* caused by chromate ions have been studied using an M-PEA-2 fluorimeter. The goal of the present work was to determine the preferred target for chromate ions action among photosynthetic reactions in marine algae and to reveal the most sensitive parameter of chlorophyll fluorescence for application in biotesting.

MATERIALS AND METHODS

The algologically pure culture of a marine unicellular diatom *P. tricornutum* isolated from the Black Sea phytoplankton and deposited in collection of the Department of Hydrobiology, Moscow State University was the subject of investigation. The culture was grown at 20 ± 2°C and illumination of 30 μE/(m^2 s) (16 h a day) in artificial sea water containing 20 g/L of salt and supplemented with (g/L): KNO_4, 0.202; NaH_2PO_4, 0.71 · 10^{-2}; $MnCl_2 \cdot 4H_2O$, 0.19 · 10^{-3}; $CoCl_2 \cdot 6H_2O$, 0.24 · 10^{-3}; $FeCl_3 \cdot 6H_2O$, 0.27 · 10^{-3} (Goldberg-Kabanova medium).

The culture of microalgae was grown for 3 days and then dispensed into 100-mL flasks. Hexavalent chrome ($K_2Cr_2O_7$ solution) was added into the liquid media in different concentrations of 2.5 to 25 mg/L (Sigma, United States). The cultures supplemented with hexavalent chrome were incubated under the same conditions as the inoculum. Prior to chromate addition, cell number in the stationary phase was 10^6 cells/mL. The number of microalgae was determined by cell count in a Goryaev chamber.

The parameters of chlorophyll fluorescence in intact cells were registered in cuvettes using an Aqua-Pen-CAP–C100 portative pulse fluorimeter (Photon Systems Instruments, Czech Republic) under illumination with red light at λ = 650 nm and 3000 μE/(m^2 s). Prior to measurements, the specimens were incubated in the dark for 30 min. Induction curves of prompt (PF) and delayed chlorophyll fluorescence (DF), as well as P_{700} redox transformations were simultaneously registered using a Multi-function Plant Efficiency Analyser (M-PEA-2, Hansatech Instruments, United Kingdom) (Strasser et al., 2010; Bulychev et al., 2013; Goltsev et al., 2014). Prompt and delayed fluorescence were registered by alternating the intervals of red light illumination (1300 μE/(m^2 s), 625 nm) and short dark intervals sufficient for detection of delayed fluorescence. DF dynamics reflected the variations in emission intensity within the range of 0.9 to 1 ms between light impulses. Absorption changes at 820 nm reflected the redox state of P_{700}, PS I reaction center (Goltsev et al., 2014). Intensity of modulating light at a wavelength of 820 ± 25 nm was 1000 μE/(m^2 s). The data obtained were normalized to the value at t = 0.7 ms (MR_0) (Goltsev et al., 2014). Characteristics and protocol of the measurements with M-PEA-2 were described in detail (Bulychev et al., 2013; Oukarroum et al., 2013; Goltsev et al., 2014). Prior to the measurements, algal cells were concentrated on membrane filters and incubated for 10 min in a wet state.

The kinetic of P_{700}^+ reduction in vivo was measured using a dual-wavelength (830 nm/900 nm) time-resolving photometer built at the Department of Biophysics of the Moscow State University (Solovchenko et al., 2015). P_{700} reduction was monitored every 0.5 ms via the difference in the intensities of light absorption at 830 nm. The specimen was illuminated with actinic light with intensity of 5000 μE/(m^2 s) for 20 ms. After cessation of the actinic illumination, kinetic of P_{700}^+ reduction by the electrons originating from PS I was registered.

To quantitatively analyze the characteristics of the primary processes of photosynthesis on the basis of the OJIP kinetic curve parameters, the JIP test was used (Lazár and Schansker, 2009; Strasser et al., 2010; Goltsev et al., 2014). The JIP test used the following parameters of the fluorescence induction kinetic curve: fluorescence intensity at 20 μs (F_O), 30 μs (F_I), 6 s (F_{6s}), as well as F_P (F_M, the maximum fluorescence yield) and M_0 (the area above the OJIP kinetic curve and below the F_M level).

These measured values were used to calculate the following parameters:

$F_V = F_M - F_O$ – maximum variable fluorescence;

F_V/F_M – maximum quantum yield of the primary photochemical reaction in open reaction centers of PS II: $F_V/F_M = \varphi_{Po}$;

φ_{Eo} – quantum yield of electron transport (t = 0): $\varphi_{Eo} = [1 - (F_O/F_M)] \cdot (1 - V_J)$ где $V_J = (F_J - F_O)/F_V$;

$DI_0/RC = (ABS/RC) - M_0\ (1/V_J)$ – total energy dissipated per reaction center (RC) as heat, fluorescence, and energy transfer to other photosystem, t = 0;

ABS/RC – energy flow absorbed per active reaction center (RC), which characterizes the relative size of the antenna (ABS): $ABS/RC = M_0/V_J\ (1/\varphi_{Po}) = (M_0/V_J)/[(F_M - F_O)/F_M)]$;

PI_{ABS} – the performance index, an indicator of the PSII functional activity relative to the absorbed energy: $PI_{ABS} = [1 - (F_O/F_M)]/(M_0/V_J) \cdot [(F_M - F_O)/F_O] \cdot [(1-V_J)/V_J]$;

q_E – ΔpH-dependent nonphotochemical quenching: $q_E = (F_M - F_{6s})/F_V$.

Absorption spectra of algal cell suspensions within the wavelength range of 400 to 850 nm were measured using a single-beam spectrophotometer equipped with a integrating sphere based on a USB2000 spectrometer (Ocean Optics, United States) (Solovchenko et al., 2015).

Measurements were performed in at least five repeats. Average values are shown on the figures.

RESULTS AND DISCUSSION

Preliminary studies demonstrated that the minimal chromate ions concentration that decreased the growth rate of marine alga *Phaeodactylum tricornutum* was 2.5 mg/L (Fig. 1). Growth of the cultures was inhibited by 50% in the presence of 5 mg/L of $K_2Cr_2O_7$. Growth inhibition by chrome salts was

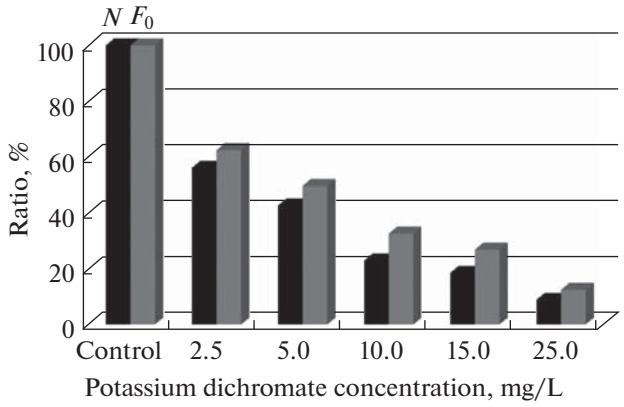

Fig. 1. Cell numbers (N) and values of fluorescence (F_O) in *P. tricornutum* microalgae after 72 h of incubation depending on $K_2Cr_2O_7$ concentration (% of the control).

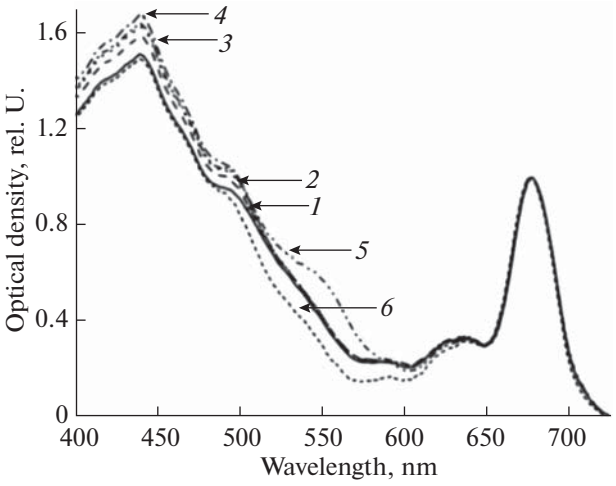

Fig. 2. Absorbance spectra of the cells of microalgae *P. tricornutum* in the presence of different $K_2Cr_2O_7$ concentrations: *1* – control and *2–6* – cells incubated for 24 h in the presence of $K_2Cr_2O_7$ (2.5; 5; 10; 15; and 25 mg/L, respectively). The spectra were normalized to absorption in the red maximum of chlorophyll a at 678 nm.

detected in various freshwater algae (Ali et al., 2006; Khalida, 2012). Decrease in growth rate and chlorophyll synthesis was demonstrated also for aquatic higher plants *Spirodela polyrhiza* and *Vallisneria spiralis* (Appenroth et al., 2001; Vajpayee et al., 2001).

It was previously shown that fluorescence intensity in marine algae (F_O) correlated with chlorophyll content and could be used as an indicator of algal abundance (Matorin et al., 2004; Matorin et al., 2012). Experimental results demonstrated that algal cell growth test-function (N) correlated with the fluorescence parameter (F_O) (correlation coefficients were at least 0.90). It confirmed the possibility of application of the fluorescence parameter (F_O) in biotesting of saline waters using the microalga *P. tricornutum* as a test organism. Application of the fluorescence parameter F_O in biomonitoring and biotesting was proposed for algal cultures and phytoplankton (Zhmur, 2009; Matorin et al., 2012).

Absorption spectra of *P. tricornutum* after incubation in the presence of different concentrations of chromate ions are shown on Fig. 2. These spectra were normalized to absorption in the red maximum of chlorophyll at 678 nm. The spectra of the control cultures were typical of diatoms with absorption maxima at 640 nm (chlorophyll *c*) and 678 nm (chlorophyll *a*) (Falkowski and Raven, 1997). Incubation in the presence of chromate ions for less than 24 h did not result in changes of absorption spectra. However, longer incubation resulted in increased absorbance in the carotenoids absorption peak (of 430 to 480 nm) that usually occurs in response to the action of stress factors (Falkowski and Raven, 1997). Amount of chlorophyll *c* decreased slightly only at high chromate concentrations (above 20 mg/L).

Fluorescent methods make it possible to obtain detailed information on disturbances of the photosynthetic apparatus at early stages of photosynthetic activity. Measurement of the ratio between the fluorescence intensity under the photosynthesis saturating illumination (F_M) and under low light intensity which induced no changes in the state of the photosynthetic apparatus (F_O) made it possible to determine the maximum efficiency of the processes in PSII, which was equal to $F_V/F_M = (F_M - F_O)/F_M$. The F_V/F_M ratio is a dimensionless energetic characteristics of photosynthesis, which is analogous to the efficiency coefficient. It was demonstrated that the maximum quantum yield of primary photochemical reactions F_V/F_M (φ_{Po}) in the control cells was high (0.612) (Table 1). In contrast to the control cells, incubation in the presence of different concentrations of chromate ions for several hours resulted in a decrease in this parameter. F_V/F_M changes occurred mainly due to the decrease in the amplitude of F_M maximal fluorescence. It should be noted that F_O and, accordingly, cell number changed insignificantly during short-term incubation (for several hours) of the algae in the presence of chromate ions. Absorbance spectra of algal suspensions almost did not change (data not shown), indicating the absence of the effect on the photosynthetic pigment apparatus. Thus, changes of photosynthetic activity (F_V/F_M ratio) make it possible to detect the effect at early stages of toxic action.

To evaluate the changes of photosynthetic activity in *P. tricornutum* cells in detail, induction curves of prompt and delayed fluorescence as well as the redox state of P_{700} were measured. Fluorescence induction kinetic curves (OJIP) normalized to F_O value are shown on Fig. 3a. The fluorescence curve of the control cells was similar to those described in the literature (Lazár and Schansker, 2009; Strasser et al., 2010). Several stages of the fluorescence induced by highly

Table 1. OJIP parameters of fluorescence kinetics of induction curves of *P. tricornutum* microalgae exposed different concentrations of chromate ions after 24 h of the incubation, % of the control

Parameter	Control	$K_2Cr_2O_7$ concentration, mg/L				
		2.5	5	10	15	25
F_V/F_M	0.612 ± 0.093 100%	0.571 ± 0.021 93%	0.539 ± 0.049 88%	0.535 ± 0.027 87%	0.529 ± 0.110 86%	0.532 ± 0.062 87%
F_V/F_O	1.575 ± 0.617 100%	1.329 ± 0.120 84%	1.168 ± 0.282 74%	1.149 ± 0.170 73%	1.123 ± 0.368 71%	1.138 ± 0.110 72%
φ_{Eo}	0.359 ± 0.013 100%	0.290 ± 0.040 81%	0.303 ± 0.039 84%	0.305 ± 0.019 85%	0.277 ± 0.040 77%	0.267 ± 0.033 74%
PI_{ABS}	0.739 ± 0.104 100%	0.397 ± 0.104 54%	0.408 ± 0.102 55%	0.401 ± 0.059 54%	0.329 ± 0.052 45%	0.299 ± 0.042 40%
ABS/RC	3.024 ± 0.272 100%	3.469 ± 0.235 115%	3.668 ± 0.261 121%	3.802 ± 0.171 126%	3.747 ± 0.851 124%	3.838 ± 4.682 127%
DI_0/RC	1.174 ± 0.453 100%	1.489 ± 0.189 127%	1.691 ± 0.244 144%	1.769 ± 0.156 151%	1.765 ± 1.154 150%	1.795 ± 4.086 153%
q_E	1.63 ± 0.013 100%	1.75 ± 0.044 107%	1.86 ± 0.021 114%	1.87 ± 0.019 114%	1.89 ± 0.039 116%	1.88 ± 0.024 115%
V_K	0.100 ± 0.056 100%	0.128 ± 0.027 127%	0.117 ± 0.031 117%	0.121 ± 0.049 121%	0.128 ± 0.051 127%	0.137 ± 0.066 137%

intensive illumination, termed O-J-I-P transients are known (Strasser et al., 2010). The initial O value corresponds to chlorophyll fluorescence intensity in open RC of PS II (F_O), when all Q_A molecules are oxidized. The O-J stage is the result of light-induced Q_A reduction, whereas the subsequent stages reflect mainly further accumulation of reduced Q_A caused by a decrease in its reoxidation due to the reduction of Q_B and the quinone pool.

To analyze quantitative characteristics of the primary photosynthetic processes based on the O–J–I–P kinetic curve, the JIP test described above was used (Fig. 3).

The amplitude of the JIP phase normalized to OJ or OP amplitude may characterize the probability of electron transport from Q_A to the plastoquinone (PQ) pool, which mainly depends on the concentration of Q_B-nonreducing RC in PS II (Strasser et al., 2010). Time required to reach the maximal fluorescence intensity P (F_M) depends on PQ pool reduction rate and also reflects the concentration of Q_B-nonreducing RC (Strasser et al., 2010). Chromate ions changed the shape of the O–J–I–P curve, while the impact of the J–I–P phase decreased, indicating disruption in the electron flow (φ_{Eo}) from PS II to the quinone pool (Table 1) (Lazár and Schansker, 2009). The quantum yield of electron transport in PS II (φ_{Eo}) decreased in the cells incubated with chromate ions. Thus, one of the first targets affected by hexavalent chrome was localized in the acceptor side of PS II. It confirmed the data obtained in the work (Prasad et al., 1991). The experiments with an exogenous electron donor for PS II and 2,6-dichlorphenolindophenol demonstrated that this site was damaged in the presence of chrome ions.

The parameter PI_{ABS} is the ratio of PS II functional activity to absorbed energy (ABS). This parameter was considerably higher in the control cells than in the cells incubated in the presence of chromate ions. Low values of the parameter PI_{ABS} (performance index) in the algal cells treated with chromate ions indicated low PS II functional activity determined mainly by a decrease in the fraction of active RC and by increased quenching of excited states in the antenna. Decrease in the efficiency of excitation energy transfer from the light-harvesting complex to RC should be accompanied by an increase in the dissipation of the unused light energy. In fact, energy dissipation efficiency (DI_0/RC) in the cells in the presence of chromate ions was indeed high. This correlated with the increase in ΔpH-dependent nonphotochemical fluorescence quenching q_E calculated as the decline in fluorescence after reaching the maximum ($q_E = (F_M - F_{6s})/F_V$). The increase in nonphotochemical fluorescence quenching in green freshwater algae was shown using a PAM (Pulse Amplitude Modulation) fluorimeter (Khalida et al., 2012).

The parameter ABS/RC in the cells incubated in the presence of chromate ions was considerably higher than in the control due to a decrease in the number of active RC. The experiments with *Lemna gibba* demon-

strated that the antenna size increased in the presence of chromate ions, which was probably caused by impaired biosynthesis of the D 1 protein (Ali et al., 2006).

The effect of heavy metals on the donor side of PS II was also demonstrated (Kumar et al., 2014). Chrome salts can affect the oxygen-evolving complex (OEC), as was shown for of duckweed (Ali et al., 2006). Decrease in the F_V/F_O parameter responsible for the change of water splitting efficiency in PS II (at the concentrations 15 and 25 mg/L) shown in the present study also indicated the possible effect of chrome compounds on this site of the OEC.

It was previously shown that under stress caused by various factors, emergence of an additional peak K was observed on the fluorescence curve around 300 μs (Lazár and Schansker, 2009; Strasser et al., 2010; Goltsev et al., 2014). It was proposed that the K peak was probably caused by damage to the donor side of PS II and was revealed by means of subtraction of the control curves from the curves with the OEC damaged by contaminants. In the presence of chromate ions, the K peak described by the parameter V_k was also observed on the fluorescence curve, indicating disruption of the water splitting process (Table 1). OEC inhibition is also able to affect electron transport in PS II.

Measurements of such values as F_V/F_M (PS II maximum quantum yield) and PI_{ABS} (performance index), indicators of the PS II functional activity, demonstrated that chromate ions caused PS II inactivation. These results confirmed the data on toxic effect of chromate ions on PS II obtained by the example of other photosynthetic objects (Hörcsik et al., 2007; Didur et al., 2013).

Millisecond DF occurs due to the secondary recombination reaction. Its intensity depends on the value of the electrochemical proton gradient across the thylakoid membrane, energy of which decreases the activation energy of reverse recombination (Matorin and Rubin, 2012; Oukarroum et al., 2013; Goltsev et al., 2014). Thus, determination of DF parameters is the method that makes it possible to monitor the changes in proton gradient across the photosynthetic cell membrane. Maximum on the DF curve within a millisecond range coincided with the J phase on the induction curve of the prompt fluorescence (Fig. 3a). Formation of this peak was caused by accumulation of emissive redox states responsible for reverse charge recombination and quanta emission (i.e., fluorescence state) as well as enhancement of delayed fluorescence due to the formation of the transmembrane electrical potential (Δψ). The DF peak within the range of seconds is associated with light-induced formation of the transmembrane proton gradient (ΔpH), which also decreases the activation energy of emissive transitions (DF emission) in PS II RC. These regularities have been considered in a number of works (Oukarroum et al., 2013; Goltsev et al., 2014;).

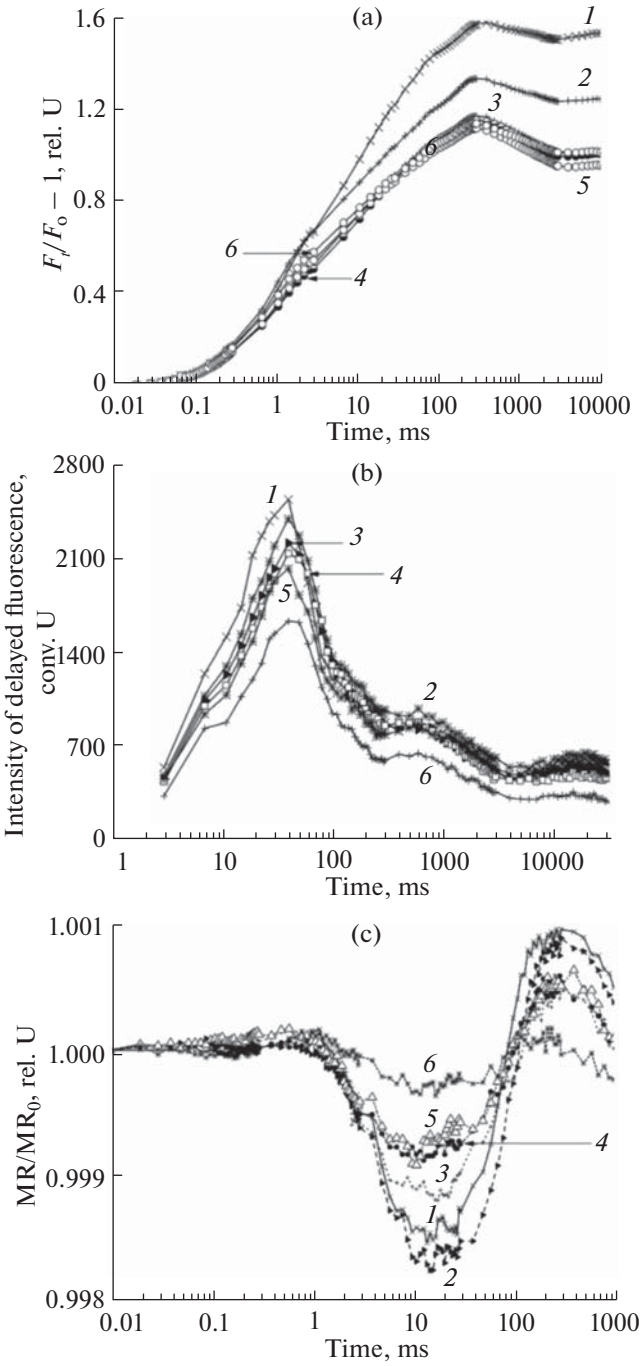

Fig. 3. Induction curves of prompt (a) and slow fluorescence (b) and changes in the reflection at 820 nm (c) in the cells of microalgae *P. tricornutum* in the presence of different $K_2Cr_2O_7$ concentrations: *1* – control, *2–6* – cells incubated for 24 h in the presence of $K_2Cr_2O_7$ (2.5; 5; 10; 15; and 25 mg/L, respectively). Prompt fluorescence was normalized to F_O. Intensity of the affecting red illumination was 1300 μE/(m^2 s). Simultaneous measurement of three parameters were performed using an M-PEA-2 fluorimeter.

DF intensity in the cells considerably decreased in the presence of chromate ions at 10–50 ms and 1 s (Fig. 3b), which was probably caused by a decrease in

Table 2. Changes of P_{700}^+ amplitude during illumination for 20 ms at 5000 μE/(m^2 s) and the amplitudes of fast and slow components of dark P_{700} reduction in *P. tricornutum* microalgae exposed to different chromate ions concentrations after 24 h of incubation, % of the control

$K_2Cr_2O_7$ concentration, mg/L	Initial P_{700}^+ amplitude after illumination	Fast component	Slow component
Control	100	100	0
2.5	108	90	10
5	87	89	11
10	88	87	13
15	87	76	24
25	70	67	33

non-cyclic electron transport and therefore decreased energization of photosynthetic membranes.

Measurement of modulated reflection at 820 nm makes it possible to monitor the reactions in P_{700} (RC of PS I). Maximum of P_{700}^+ accumulation was observed at $t \approx 30$ ms (MR_{min}) and then was replaced with P_{700} reduction (Fig. 3c). The fluorescence signals reflecting Q_A and P_{700} reduction reached a plateau almost synchronously. Simultaneous accumulation of reduced P_{700} and Q_A reflects reduction of the carriers throughout the electron transport chain between the photosystems due to the absence of electron outflow from the acceptor side of PS I under conditions of inactivation of ferredoxin-NADP reductase (FNR) due to the incubation in the dark. Long illumination (of 1 to 10 s) resulted in the second wave of P_{700} oxidation that may be explained by electron outflow from PSI due to activation of FNR and the Calvin cycle enzymes.

P_{700} in algal cells exposed to low concentrations of chromate ions retained the capability for oxidation after illumination switch (Fig. 3c). However, the rate of P_{700} reduction by PS II decreased. It was consistent with the results of analysis of induction curves of prompt fluorescence. Higher concentrations caused considerable inhibition of P_{700} oxidation in PS.

Kinetics of dark P_{700}^+ reduction after far-red illumination (see Materials and Methods) was studied to analyze the effect of chromate ions on PS I reactions in detail. The P_{700}^+ signal was accumulated during illumination of the culture with actinic light (700 nm) for 20 ms. Chromate ions at low concentration (up to 5 mg/L) did not affect signal accumulation (Table 2). In the presence of chromate ions in concentrations exceeding 5 mg/L, P_{700}^+ oxidation amplitude decreased.

After cessation of actinic illumination, dark P_{700}^+ reduction occurred (Fig. 4). In untreated culture, P_{700}^+ reduction was performed due to the influx of electrons from PS II, as well as to additional sources from cyclic electron transport and stromal donors (Wodala et al., 2012). The P_{700}^+ reduction kinetic curves in the algae were decomposed into two components of the exponent: the fast component of P_{700}^+. reduction with the time of ~0.5 ms, which reflects donation from plastocyanin in noncyclic electron transport, and the slow component of P_{700}^+ reduction with time constant of 15 ms. The slow component may reflect P_{700}^+ reduction from both noncyclic and cyclic electron transport (Hope, 2000; Solovchenko et al., 2015).

The rate of P_{700}^+ reduction in the presence of chromate ions decreased after cessation of the illumination, which was in agreement with the data on the rate of P_{700}^+ reduction obtained using M-PEA-2 fluorimeter. The data shown on Fig. 4 and in Table 2 demonstrate that the amplitude of the fast stage of P_{700}^+ signal recovery decreased. This, in turn, indicated a decrease in electron flow from PS II to P_{700}^+. It should be noted that the amplitude of the slow phase increased with increasing concentration of potassium dichromate, probably indicating an increase in the rate of cyclic electron transport. It is a typical response to a range of stress factors (Solovchenko et al., 2015). The very slow stage, i.e. electron flow from stromal donors to PS I, probably also contributed toP_{700}^+ reduction. This was previously described in the study on the effect of copper on pea leaves (Wodala et al., 2012).

It is known that photoinhibition of photosynthesis can occur due to enhanced light intensity. In this case, protective processes of non-emissive dissipation of excess light energy are activated. It was shown that photoinhibition is mainly associated with degradation of the D 1 protein, which is encoded in the chloroplast genome and is one of the main PS II components (Chow and Aro, 2005).The recovery of PS II activity is associated with re-synthesis of this protein in chloroplasts. It is obvious that the concentration of active

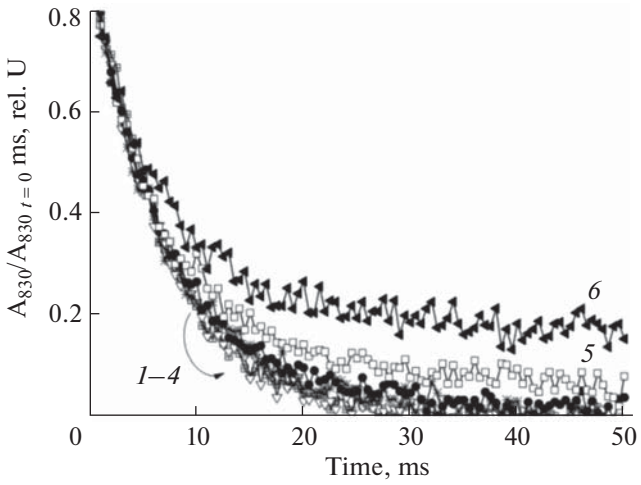

Fig. 4. The effect of $K_2Cr_2O_7$ on kinetics of the dark P_{700}^+. reduction after illumination of *P. tricornutum* microalgae for 20 ms at 5000 μE/(m^2 s): *1* – control, *2–6* – cells incubated for 24 h in the presence of $K_2Cr_2O_7$ (2.5; 5; 10; 15; and 25 mg/L, respectively).

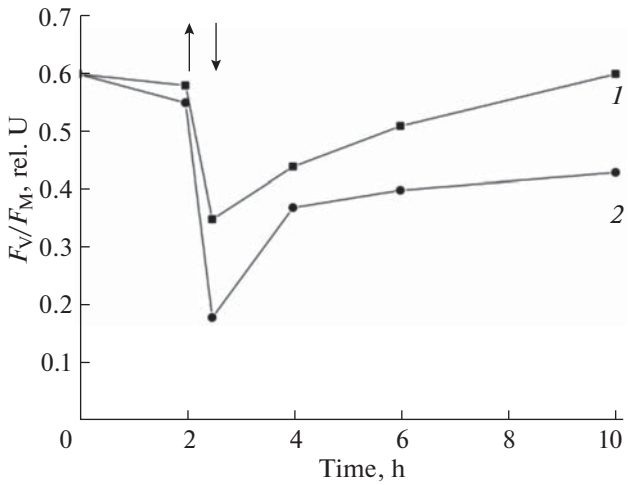

Fig. 5. Change of the F_V/F_M value in cell suspension of *P. tricornutum* during the incubation under intensive illumination (500 μE/(m^2 s)) for 25 min and a subsequent dark period: *1* – control, *2* – cells incubated in the presence of $K_2Cr_2O_7$ (5 mg/L). Intensive illumination was switched 20 min after the addition of the chrome salt. Up and down arrows switching the light on and off, respectively.

PS I centers in the cells depends on the ratio between the rates of its photooxidative destruction and reparation, which may be detected as a decrease in the F_V/F_M value under intensive illumination and its subsequent reparation in the dark, respectively (Vavilin et al., 1995; Pätsikkä et al., 1998). Photoinhibition of photosynthesis caused by photooxidative stress in the cells exposed to intense illumination may be enhanced due to different unfavorable factors including the toxic action of heavy metals (Vavilin et al., 1995; Chow and Aro, 2005).

Changes of the F_V/F_M value in the culture *P. tricornutum* sequentially incubated under enhanced illumination and in the dark are shown on Fig.5. In the samples treated with low concentrations of chromate ions, photoinhibition considerably enhanced and the rate of dark recovery of the F_V/F_M value decreased compared to the control (Fig. 5). These results indicated that chromate ions probably inhibited the reparation of light-damaged PS II centers in algal cells. Decrease in the F_V/F_M value in the presence of chromate ions was probably caused by their effect on the biosynthesis of the D 1 protein. The experiments with duckweed *Lemna gibba* directly demonstrated a decrease in D 1 content in the presence of chromate ions (Ali et al., 2006). It may play an important role in decrease in the photosynthetic activity under the stress conditions caused by low concentration of these metals. The same study showed that hexavalent chrome salts caused emergence of reactive oxygen species destroying the lipids of the thylakoid membrane and thus causing destruction of the membranes and damage of the protein complex structure (Ali et al., 2006).

CONCLUSION

The data obtained demonstrated that chromate ions considerably inhibited the growth rate of marine microalgae *P. tricornutum*. The correlation revealed between directly counted cell numbers and the numbers determined by means of measurement of the fluorescence parameter F_O confirmed that fluorescence measurement may be a promising method in biotesting instead of the time-consuming and labor-intensive technique of direct cell count under a microscope.

Simultaneous measurements of the kinetics of prompt and delayed fluorescence as well as of the P_{700} redox state using an M-PEA-2 fluorimeter made it possible to reveal the early stages of chromate ions action on photosynthetic reactions in marine algae. Induction curves analysis demonstrated that one of the action sites of hexavalent chrome was localized on the acceptor side of PS II between Q_A and Q_B. Decreased quantum yield of electron transport in PS II resulted in a decrease in performance index (PI_{ABS}). This parameter was more sensitive than F_V/F_M and may be recommended for application in biotesting.

Decrease in the number of active RC and change of electron transport resulted in increase in non-photochemical losses associated with the increase in quantum efficiency of energy dissipation (DI_0/RC) and ΔpH-dependent nonphotochemical quenching (q_E).

Analysis of P_{700} redox state (PS I) revealed that P_{700} oxidation was less sensitive to hexavalent chrome compounds. Analysis of dark P_{700} reduction kinetics

demonstrated inhibition of the fast phase of electron donation from plastocyanin and PS II. At the same time, the slow phase associated with cyclic electron transport and stromal acceptors increased. The effect of high chromate concentrations on the donor side of PS II indicated the possibility action of hexavalent chrome on this site of OEC.

Enhancement of photoinhibition and deceleration of reduction reactions in the dark after cessation of the photooxidative stress in *P. tricornutum* cells in the presence of chromate ions confirmed the possible effect of chromate ions on the biosynthesis of the PS II D 1 protein.

Thus, the results of our study revealed that the change of induction curves of prompt and slow fluorescence is one of the first rapidly registered parameter of microalgae cells exposed to the action of hexavalent chrome compounds. These parameters may be efficiently applied in diagnostic of the state of the studied objects. The parameters may also be useful for application of chlorophyll fluorescence of microalgae in biotesting of water quality in natural and artificial reservoirs.

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