PHOTOSYNTHETICA
International Journal for Photosynthesis Research

DOI 10.32615/ps.2025.038 PHOTOSYNTHETICA 64 (1): 12-25, 2026

PERSPECTIVE

A reexamination of the Kitajima and Butler (1975) model for relating chlorophyll *a* fluorescence to photochemistry

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Abstract

The main assumptions of the well-known Kitajima and Butler (1975) model, describing the relationship between the ratio of the maximum variable chlorophyll *a* fluorescence to the maximum fluorescence (F_V/F_M) and the photochemical quantum yield of PSII (Φ_{Po}), have been analyzed. Using the experimental data from the literature, potential "weak points" of this model are discussed, as well as the reasons for the differences between the F_V/F_M values and the actual Φ_{Po} values. Special attention is focused on the fluorescence measurement procedures using the saturating single turnover light flashes and the saturating multiple turnover light pulses. It is concluded that if the F_V/F_M measurements are made properly, the value of Φ_{Po} can indeed be estimated.

Keywords: chlorophyll *a* fluorescence; multiple turnover; photochemical quenching; quantum yield; saturating flash; single turnover; variable fluorescence.

Introduction

A phenomenon largely used in the study of photosynthesis is Chl *a* fluorescence induction, which takes place during a dark-to-light transition in oxygenic photosynthetic organisms, and was discovered by Kautsky and Hirsch (1931). They observed with their own eyes the Chl *a* fluorescence changes taking place for several minutes: first, there was a fast increase in fluorescence, and then a slow decrease to a minimum. Kautsky and Hirsch (1931) discussed these transient Chl fluorescence changes in relation to processes in photosynthesis (*see* also *e.g.*,

Highlights

- On the assumptions of the Kitajima and Butler (1975) chlorophyll fluorescence emission model
- Using proper F_V/F_M measurements, the quantum yield of PSII can indeed be assessed
- The influence of the technical features of the fluorometer(s) on the measured F_V/F_M ratio is considered

Received 5 September 2025
Accepted 12 December 2025
Published online 12 January 2026

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Abbreviations: DBMIB – dibromothymoquinone; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ETR – electron transport rate; F_M – maximal chlorophyll (Chl) *a* fluorescence yield; F_M' – maximal Chl *a* fluorescence yield in light-adapted state; F_O – minimal Chl *a* fluorescence yield in dark-adapted state; F_S – steady-state fluorescence yield in light-adapted state; $F_V = F_M - F_O$ – maximal variable fluorescence; HIQ – high-intensity quenching; MT – multiple turnover; NPQ – nonphotochemical quenching; P_{680} – primary (electron) donor of PSII; Phe – pheophytin, the primary (electron) acceptor of PSII; PQ-pool – plastoquinone pool; RC – reaction centre; ST – single turnover; Φ_{Po} – maximum photochemical quantum yield of PSII photochemistry; Φ_{PSII} – effective quantum yield of PSII photochemistry in light-adapted state.
Acknowledgements: We thank Robert Blankenship for reading an earlier draft of this paper. G. Govindjee is thankful to the staff of the Information Technology, Life Sciences, University of Illinois at Urbana-Champaign, for their help.
Dedication: In memory of Natalia Belyaeva (1945–2025) – an outstanding researcher in the field of computer simulation of primary photosynthesis and light-induced kinetics of chlorophyll fluorescence.
Conflict of interest: The authors declare that they have no conflict of interest.

Govindjee 1995, Schreiber and Lichtenthaler 2025). [For the interpretation of Chl induction curves, *see* Stirbet *et al*. 2014; and chapters in books edited by Papageorgiou and Govindjee (2004) and Suggett *et al*. (2010)].

When measuring Chl *a* fluorescence induction with a *Plant Efficiency Analyzer* (PEA) fluorometer with a saturating continuous multiple turnover (MT) light [1,500–3,000 µmol(photons) m^{-2} s^{-1}], the initial fast (< 1 s) Chl fluorescence increase is known as the OJIP fluorescence transient. The OJIP curve is multiphasic, which is better observed when it is plotted on a logarithmic time scale: the fluorescence increases from an initial value F_O (at point O), to a maximum value F_M (at point P), and between O and P there are two inflections, which are observed at ~ 2 ms (point J) and at ~ 30 ms (point I), where the respective fluorescence values are F_J and F_I. The O-J fluorescence increase is known as the "photochemical phase", since its relative height and initial slope depend on the number of photons absorbed by the sample per unit of time (which is proportional to the irradiance and PSII absorption cross section), and is not very sensitive to temperature. The J-I-P fluorescence increase is also called the "thermal phase", which is correlated with the photoreduction by PSII of the plastoquinone pool in the thylakoid membrane, is sensitive to temperature variations (disappearing at subfreezing temperatures), and is less affected by changes in light intensity (*see* discussion in Stirbet *et al*. 2014).

On the other hand, when the Chl fluorescence transient is measured with a Fast Repetition Rate (FRR) fluorometer, after applying a saturating single-turnover (ST) sequence of µs light flashes [of ~ 20,000 µmol(photons) m^{-2} s^{-1}], fluorescence increases from a minimum value of F_O to a maximum value of F_M in 30–50 µs. However, the measured F_M/F_O ratios using ST light flashes are lower than those obtained from Chl fluorescence measurements with MT light (*see* above), and this depends on the duration of the light flashes (France *et al*. 1992).

The most used fluorescence parameter calculated from the fluorescence induction data is the F_V/F_M ratio, where $F_V = F_M - F_O$ is the maximal variable fluorescence (*see e.g*., Stirbet and Govindjee 2011).

In different fluorescence studies on various plants illuminated with MT light, it has been shown that the observed F_V/F_M values positively correlate with the photochemical yield of PSII (Φ_{Po}), which has been independently estimated by using other types of measurements, such as: (i) the C-550 signal (*see* explanation in the next section; *cf.* Kitajima and Butler 1975); (ii) the rates of oxygen evolution on the electron donor side of PSII (*e.g*., Björkman and Demmig 1987); (iii) picosecond Chl fluorescence kinetics measured on isolated pea chloroplasts (*e.g.*, Roelofs *et al.* 1992); and (iv) time-resolved fluorescence technique on thylakoid membranes of *Arabidopsis thaliana* (*e.g.*, Wientjes *et al.* 2013).

Further, we note that the effective quantum yield of PSII photochemistry, under light-adapted conditions, $\Phi_{PSII} = (F_M' - F_S)/F_M'$, also known as the Genty parameter (Genty *et al*. 1989), is connected with the F_V/F_M measured under dark-adapted conditions by using Pulse-Amplitude Modulation (PAM) fluorometry (*see* Porcar-Castell *et al*. 2014). The Φ_{PSII} values have also been verified on plants through parallel measurements of CO_2 assimilation rates under low photorespiration conditions (*e.g.*, Genty *et al*. 1989), or the rates of oxygen evolution (*e.g.*, Öquist *et al*. 1992). On the other hand, Φ_{PSII} has also been used by many from measurements using Fast Repetition Rate (FRR) fluorometers (*e.g*., Oxborough *et al*. 2012, Boatman *et al*. 2019, Schuback *et al*. 2021) to indirectly estimate the rates of photosynthesis in algal cultures and the primary production of phytoplankton by measuring the slope of the linear electron transport rate (ETR) as a function of light intensity. In comparison with the direct methods for measuring the rate of photosynthesis (*i.e.*, by recording the oxygen evolution or the CO_2 uptake), the indirect ETR quantification by means of Φ_{PSII} measurements is faster. It has high sensitivity at low Chl content in organisms growing in natural waters (Antal *et al.* 2001a,b). Some studies have shown good correlation between the direct methods of measuring primary production and ETR values, while others have not. A review of the relevant work is available in Perkins *et al.* (2010).

Below, we examine, in detail, the model of Kitajima and Butler (1975) on the relation between the Chl *a* fluorescence and the PSII photochemistry.

The theoretical model of Kitajima and Butler (1975)

The physical meaning of the F_V/F_M in dark-adapted samples, *i.e.*, as a proxy of the maximum photochemical quantum yield of PSII, Φ_{Po}, was shown by Kitajima and Butler (1975) through a simple physical model, which they had also verified experimentally. [Warren Butler's scientific biography is available in Benson (1998), and his papers are listed in Govindjee (1986)]. We summarize below the basics of the above-mentioned model.

Kitajima and Butler (1975) have analyzed the quenching of both fluorescence and photochemistry in terms of the rate constants for the depopulation of the first excited singlet state of Chl by fluorescence (k_F), nonradiative internal decay processes (k_D), photochemistry (k_P), and by the specific quenching (k_Q) by DBMIB. They made such an analysis by considering two types of light energy-harvesting processes: (*1*) "*independent photosynthetic units with one reaction center per unit and no energy transfer between them*", and (*2*) "*complete energy transfer between the units; essentially a matrix of reaction centers that can exchange excitation energy between them*". The second model is also known as the "lake" model (Robinson 1966). Since both the models arrive at the same expressions for the quantum yields of fluorescence and photochemistry at the initial and final fluorescence states (represented by F_O and F_M measured at low temperature), for simplicity, we present below only the equations for the lake model.

The quantum yield of a concurrent process can be calculated as the ratio between the rate constant of that process and the sum of the rate constants of all the processes. Kitajima and Butler (1975) wrote

the following equations (1–3) of the quantum yields for F_O (Φ_{Fo}), of PSII photochemistry (Φ_{Po}), and for the maximum fluorescence F_M (Φ_{FM}), all measured in the presence of different concentrations of an external fluorescence quencher Q (in their experiment, it was dibromothymoquinone).

$$\Phi_{Fo} = \frac{k_F}{k_F + k_D + k_Q + k_P} \quad (1)$$

$$\Phi_{Po} = \frac{k_P}{k_F + k_D + k_Q + k_P} \quad (2)$$

$$\Phi_{FM} = \frac{k_F}{k_F + k_D + k_Q} \quad (3)$$

Note that, in equation (3), at the moment when the Chl fluorescence reaches its maximum value F_M (when all active PSII RCs are closed), the $[Q_A] = 0$, and thus $k_P = 0$.

Therefore, if we note the absorbed light intensity by PSII to be J_{abs}, the fluorescence values of F_O and F_M can be calculated as:

$$F_O = J_{abs} \times \frac{k_F}{k_F + k_D + k_Q + k_P}$$

and

$$F_M = J_{abs} \times \frac{k_F}{k_F + k_D + k_Q} \quad (4)$$

Finally, based on the above equations, it can be shown that F_V/F_M equals Φ_{Po}:

$$\frac{F_V}{F_M} = \frac{\Phi_{FM} - \Phi_{Fo}}{\Phi_{FM}} = \frac{k_P}{k_F + k_D + k_Q + k_P} = \Phi_{Po} \quad (5)$$

At the time of publication of the Kitajima and Butler (1975) paper, the presence of the electron carrier pheophytin between P_{680} and quinone Q_A was not known, although there was some experimental data in favor of the "lake" model (Briantais *et al.* 1972, *see* also Malkin *et al.* 1980). Kitajima and Butler (1975) found that the F_V/F_M ratio is a proxy of Φ_{Po} independently of whether the PSII units are energetically connected or not. Indeed, both F_O (when all the active PSII RCs are open) and F_M (when all the active PSII RCs are closed) do not depend on PSII connectivity (*see* also Björkman and Demmig 1987, Dau 1994, Lavergne and Trissl 1995, Porcar-Castell *et al.* 2014).

To demonstrate the correctness of the equation (5), Kitajima and Butler (1975) made parallel measurements of both the C-550 signal, and the Chl fluorescence induction curves (intensity of fluorescence at 690 nm as a function of time following the onset of blue exciting light) on isolated chloroplasts at –196°C (77 K), as a function of the DBMIB concentration. We note that the C-550 signal is related to a pigment whose light-induced absorbance changes reflect the primary photochemical activity of PSII, being associated with the photoreduction of Q_A (*see* Kitajima and Butler 1973, Melis and Schreiber 1979).

The yield for the quenching of the excited Chl by a specific concentration of DBMIB would be:

$$\Phi_Q = \frac{k_Q}{k_F + k_D + k_Q + k_P} \quad (6)$$

From equation (5), and by using the index Q_O in the absence, and Q in the presence of the quencher DBMIB, Kitajima and Butler (1975) obtained the following equation:

$$\left(\frac{F_V}{F_M}\right)_{Qo} - \left(\frac{F_V}{F_M}\right)_{Q} = \frac{k_P k_Q}{(k_F + k_D + k_P)(k_F + k_D + k_Q + k_P)} =$$

$$= (\Phi_{Po})_{Qo} \Phi_{Qo}$$

or

$$\Phi_Q = \frac{\left(\frac{F_V}{F_M}\right)_{Qo} - \left(\frac{F_V}{F_M}\right)_{Q}}{\left(\frac{F_V}{F_M}\right)_{Qo}} = \frac{(\Phi_{Po})_{Qo} - (\Phi_{Po})_{Q}}{(\Phi_{Po})_{Qo}} \quad (7)$$

With equation (7), Kitajima and Butler (1975) were able to determine the Φ_Q for various concentrations of DBMIB from both the F_V/F_M values and the initial rates of photoreduction of C-550.

An excellent experimental verification of the equation (5) was shown in the Fig. 4 of Kitajima and Butler (1975), as they found a very good correlation between Φ_{Po} (determined from the initial rates of C-550 photoreduction) and the F_V/F_M (calculated from the fluorescence data obtained at $\lambda = 690$ nm and –196°C, with blue MT light for excitation) for varying degrees of quenching by DBMIB.

As mentioned in the Introduction, many authors have confirmed the results of Kitajima and Butler (1975) by measuring the F_V/F_M ratios and comparing them with the quantum yields of O_2 evolution, or with the CO_2 assimilation rates under conditions of low photorespiration. Here we emphasize the research of Björkman and Demmig (1987) on leaves of 44 species of vascular plants, in which the F_V/F_M ratios were measured under similar conditions as those used by Kitajima and Butler (1975) (but without DBMIB), and a very good correlation was found between these values and the quantum yields of O_2 evolution at saturating $[CO_2]$. Furthermore, Björkman and Demmig (1987) also showed that high-light treatment of shade leaves caused a reduction in both the quantum yields of O_2 evolution and their respective F_V/F_M values, which increased with the time of exposure to high light. A linear relationship was observed when the photon yields of O_2 evolution were plotted against the F_V/F_M values. It was concluded that the measurements used in this study serve as an excellent quantitative measure of photoinhibition of overall photosynthetic energy conversion and of the photochemistry of PSII, respectively. Besides the above results, Öquist and Chow (1992) found that the maximum values of the quantum yield of PSII electron transport (Φ_{PSII}) in light-adapted samples and the photosynthetic O_2 evolution decrease in proportion to the degree of photoinhibition.

We also note that Kitajima and Butler (1975) had made an important observation, based on their measurements, that the major part of the F_O fluorescence is of the same type as the F_V fluorescence, both emanating from the bulk chlorophyll of PSII, while before their paper, it was considered that the F_O is "dead fluorescence", or a fluorescence of constant yield emanating only from PSI (*see* page 113 in Kitajima and Butler 1975, and Etienne *et al.* 1974). This observation was confirmed, based on the fact that at –196°C the entire PSII fluorescence is increased (*see e.g.*, Dekker *et al.* 1995), while the PSI fluorescence yield is decreased at wavelengths shorter than 700 nm, and the long-wavelength fluorescence of PSI is increased (*see e.g.*, Croce *et al.* 1996), so that the vast majority of the Chl fluorescence measured at $\lambda = 690$ nm and at –196°C indeed originates from PSII (*see* also the discussion on page 188 in Pfündel 1998).

However, in general, when Chl fluorescence induction is measured at room temperature, the F_V/F_M depends on the technical characteristics of the fluorometer, the type of photosynthetic organism, and its structural properties, *e.g.*, the PSI/PSII ratio. For example, Pfündel (1998) showed that, for a C_3 plant with $F_V/F_M = 0.83$ (measured at $\lambda > 700$ nm with an MT light pulse, and at room temperature), the F_V/F_M value, corrected for PSI fluorescence, is 0.88; the F_V/F_M values, corrected for PSI fluorescence, are larger than those without correction since the PSI contribution to F_O is much higher than it is for F_M. On the other hand, the F_V/F_M measured using µs ST light flashes (at $\lambda > 700$ nm at room temperature) is smaller (~ 0.67), and after correction, it increases to 0.74; (*see e.g.*, discussion in Stirbet and Govindjee 2012).

Now, we ask the question: Is the equation (5), *see* above, really valid for the photosynthetic samples under the experimental conditions that we now use? This remains to be examined since quite often the quantum yield of PSII measured, as shown above, does not agree with the actual measured yield (van Wijk and van Hasselt 1990, Tsuyama *et al.* 2003, Earl and Ennahli 2004). Indeed, when it is not ensured that the experimental extremes correspond to the real values of F_O and F_M (*i.e.*, when all the active PSII RCs are open or closed, respectively), and the Chl fluorescence is mainly PSII fluorescence (*i.e.*, with a very low contribution of PSI fluorescence or of other pigments; *see* Pfündel 1998), the calculated F_V/F_M ratio is only an "apparent" Φ_{Po}. Thus, we examine here the general assumptions in the Kitajima and Butler (1975) model.

General assumptions of the Kitajima and Butler (1975) model

When measuring Chl *a* fluorescence in plants and green algae, the contribution of PSI has only a moderate effect on the F_V/F_M values (*see* Pfündel 1998). We also note that the F_M can be affected by variable PSI fluorescence, which can be as high as 8–17% of the overall maximal fluorescence originating from both the photosystems, as first suggested by Lazár (2013). Later, this was confirmed in Chl fluorescence studies on green algae and cyanobacteria, by using different wavelengths for excitation, and by measuring fluorescence at $\lambda > 700$ nm (Schreiber and Klughammer 2021, Schreiber 2023). Still, the uncorrected F_V/F_M ratios are often used in these organisms, especially in comparative studies, since their PSI/PSII ratios are close to 1, when grown under "standard" white light conditions. However, the cyanobacteria and the red algae are different, since there the contribution of PSI to overall fluorescence is quite high (due to a larger PSI/PSII ratio); also, the overlap by the fluorescence of phycobilins (phycoerythrin, phycocyanin, and allophycocyanin) must not be neglected. However, we note that there are experimental solutions that can deal with these types of problems (*see e.g.*, Ogawa *et al.* 2017, Stirbet *et al.* 2019).

The measuring system, used by Kitajima and Butler (1975), recorded the fluorescence intensity during continuous excitation-light exposure. We note that in a dark-adapted sample all the Chl molecules are in the ground state (upper left diagram in Fig. 1). The fluorescence intensity is proportional to the number

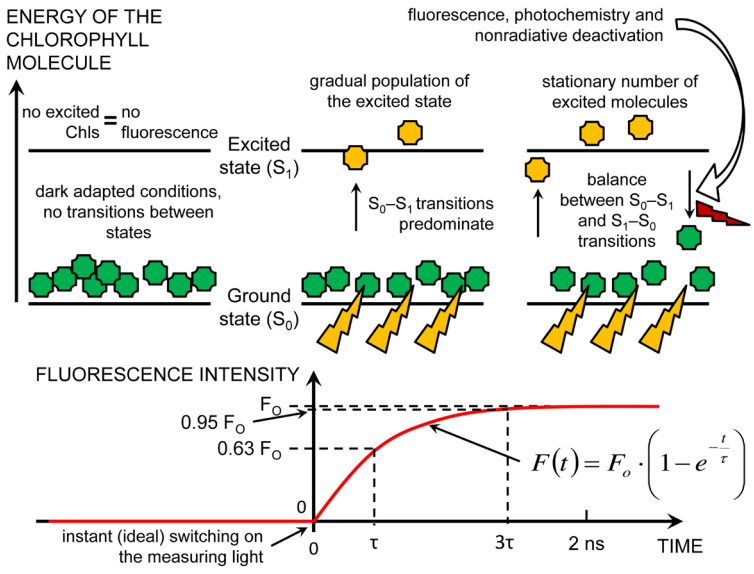

Fig. 1. A scheme for the process of initial fluorescence changes that occur immediately after switching on the weak measuring excitation light of a constant intensity. The green (round) boxes – the chlorophyll molecules in the ground (nonactivated) state, and the brown boxes – the excited chlorophyll molecules. The yellow flashes – continuous measuring light; the red flash – fluorescence emission. The F(t) curve is the overall fluorescence on a nanosecond time scale.

of radiative transitions, per unit time, from S_1, the first excited state, to the ground state S_0, and this, in turn, is proportional to k_F and to the concentration of the excited Chl molecules in the light-collecting antenna as well as in the reaction centers. Thus, after turning on the weak measuring excitation light, the fluorescence signal increases from zero to a certain stable value F_O.

In the initial excitation phase (*see* the upper middle diagram in Fig. 1), the number of upward transitions S_0–S_1 (ground state to the first excited state due to light absorption) per unit time significantly exceeds the rate of the reverse transitions. Therefore, the concentration of the excited Chl molecules as well as the fluorescence intensity continuously increases. This process has been studied on a wide time scale (from 1 ps to 1 s) and has been theoretically examined by Lazár (2003) based on a very detailed model of the PSII reactions.

Reliable determination of the F_O level becomes possible only when the fluorescence intensity becomes stable, that is, when the rates of direct and reverse transitions between the S_0 and S_1 states become equal (*see* the upper right diagram in Fig. 1). The kinetics of fluorescence transient from zero towards a stable value, which corresponds to F_O when a weak measuring excitation light is applied (lower diagram in Fig. 1), is described below in equation (8), with a saturating monoexponential curve F(t).

$$F(t) = F_O \times \left(1 - e^{-\frac{t}{\tau}}\right) \tag{8}$$

where τ is the overall Chl fluorescence lifetime.

For the derivation of the steps leading to equation (8), *see* Appendix; as shown there, when the sum of k_F, k_D, k_P, and k_Q increases, the value of F_O decreases, but the kinetics of fluorescence transient from zero towards a stable value becomes more rapid (*i.e.*, a decrease in τ). We also note that since the fluorescence intensity is proportional to the number of Chl molecules in the S_1 state, fluorescence lifetime is equal to the lifetime of the excited S_1 state. The fluorescence lifetime can be measured directly and, thus, serves as an estimate of the lifetime of the excited state. Both depend on the redox state of Q_A: the smaller the amount of oxidized Q_A, the smaller the expected value of k_P and the longer the time τ will be [*see* equation (17) in Appendix].

From the basic properties of a monoexponential function, we know that 95% of the fluorescence transient is completed within the time equal to 3τ (*see* the lower part of the diagram in Fig. 1). Thus, in practice, a time interval of 3–4 times longer than the fluorescence lifetime is sufficient to obtain the initial F_O value. The Chl fluorescence lifetime is known to be 0.1–2 ns (*see e.g.*, Briantais *et al.* 1972). The longest τ values (2 ns or more) are found for pigments in solvents where k_P is equal to zero. Thus, a stationary fluorescence level of F_O may be sampled 10 ns after switching on the measuring light (3 ns for active PSII, *see* Lazár 2003).

The real time resolution for measuring the initial fluorescence F_O varies depending on the fluorometers used: (*1*) with Pulse-Amplitude Modulation (PAM) fluorometers (Schreiber *et al.* 1986, Schreiber 2004), F_O is determined by applying a weak modulated measuring light on dark-adapted samples, and the measurement takes typically 20 or 50 μs; (*2*) with *Plant Efficiency Analyzer* (PEA) fluorometers (Strasser and Govindjee 1992), F_O corresponds to the initial fluorescence value at the "O" step of the OJIP transient, which is typically measured at 20 or 50 μs after the onset of illumination with constant light of the dark adapted sample; and (*3*) with Fast Repetition Rate (FRR) fluorometers (Kolber *et al.* 1998), F_O is sampled at the end of the first microsecond-scale light flash (0.125–1.0 μs, *see* Kolber *et al.* 1998), which is at the beginning of the saturating ST flash sequence.

We note that the steady-state concentration of the excited Chl molecules, and hence the intensity of Chl *a* fluorescence, depends on the ratio of the rate constants of the following competing processes (first-order reactions, *see* equation (1), and Kitajima and Butler (1975); as well as Fig. 3 and the related text in Lazár 2003): (i) radiative transition, k_F; (ii) photochemical quenching, k_P; (iii) nonradiative (thermal) deactivation, k_D; and (iv) additional thermal deactivation in the presence of external quenchers (nonphotochemical quenching), k_Q. In theoretical papers by Schatz *et al.* (1988) and Dau (1994), the Chl transition to a triplet excited state, with its rate constant, has also been considered.

Furthermore, in the Kitajima and Butler (1975) model, the following assumptions were made: (*1*) after dark adaptation, all the active PSII RCs are open; (*2*) at the moment when the maximum Chl *a* fluorescence is measured, all the active PSII RCs are closed, and, thus, $k_P = 0$; (*3*) the k_F, k_D, and k_Q values remain the same in the dark and under light saturation when the F_O and F_M levels are measured.

In another paper, Butler and Kitajima (1975) had emphasized that in some experiments, when the PSII activity is artificially inhibited and then restored (*see e.g.*, Malkin and Jones 1968, Yamashita and Butler 1968, 1969), the F_V/F_M values correlate very weakly with the actual photochemical activity of the PSII. To explain these facts, an extended model was proposed (Butler and Kitajima 1975), in which both the radiative and the nonradiative transitions in the antenna chlorophyll molecules, and in the chlorophylls of the RC, were considered separately, and a time-limited excitation trapping between the antenna chlorophylls and the RC chlorophylls was considered. However, this model has not been used by most researchers, as the other model proposed in Kitajima and Butler (1975) was – this may have been since it was a particular case, more complex. In addition to the concerns raised above, we now also consider the following points of view.

A complex photochemical quenching mechanism – reversible radical pair

In the Kitajima and Butler (1975) model, all the processes of deactivation of the excited state of Chl are considered as irreversible first-order reactions. If this is the case, then the kinetics of fluorescence decay in response to a single picosecond-width laser exciting pulse would represent

a single-exponential curve with a characteristic time τ (the overall Chl fluorescence lifetime):

$$\tau = \frac{1}{k_F + k_D + k_Q + k_P} \quad (9)$$

A similar Chl *a* fluorescence decay pattern can also be measured after dark adaptation of the photosynthetic sample, corresponding to the condition for F_O (*i.e.*, when all the active RCs are open). The characteristic time of this fluorescence decay (τ_{Fo}) is in the range of hundreds of picoseconds (Haehnel *et al*. 1982, Karukstis and Sauer 1983). More strictly speaking, the Chl fluorescence decay also contains a short component from PSI with a characteristic time of 40–80 ps (*see* a review by Govindjee and Satoh 1986). Also, for PSI Chl fluorescence components during the Chl fluorescence induction, *see* Lazár (2013), Schreiber and Klughammer (2021), and Schreiber (2023). But we will not discuss it further and will focus only on the PSII fluorescence components.

Under light saturation of photosynthesis, and in the presence of DCMU (dichlorophenyl dimethyl urea – a PSII acceptor side inhibitor), the value of k_P decreases. As follows from equation (9), there is an increase in the lifetime of fluorescence, although the single-exponential shape of the decay curve is expected to remain as such. However, as shown in Volgusheva *et al.* (2007), when all the RCs are closed, the fast (100–500 ps) component is retained in the decay curve, and a second, "slow" (up to 2 ns) component appears (also *see* Malkin *et al.* 1980, Haehnel *et al*. 1982, Schatz *et al*. 1988).

The appearance of a new "slow" component while a "fast" one is retained, can be described by a much more complex scheme (Schatz *et al.* 1988; *see* also a discussion in Stirbet and Govindjee 2012, and Lazár 1999, 2003), which postulates the formation, at the RC of PSII, of the radical pair [$P_{680}^+Phe^-$] (Fig. 2, k_1) and the possibility of its sufficiently rapid recombination leading to the regeneration of the excited state (*see* the rectangular box on the left in Fig. 2, k_{-1}). The value of k_2 is different for the open and closed RCIIs; further, it has, in addition, a different meaning within the reversible radical pair model in the two cases. An increase in Chl *a* fluorescence intensity from F_O to F_M corresponds to an increase in the area under the fluorescence decay curve in response to a single picosecond-width light pulse. This increase occurs due to the rise in the area under the "slow" component (due to recombination of charges in the radical pair, *see* Volgusheva *et al.* 2007); note that this is accompanied by an energy loss through k_d (Fig. 2). Due to the energy loss, the quantum yield of Chl *a* fluorescence can never reach the high values known for Chl solutions in organic solvents (10–33% for different solvents; *see e.g.*, Förster and Livingston 1952, Rabinowitch 1956).

A theoretical study of a similar model was published by Trissl *et al.* (1993), which showed that different sets of parameters (kinetic constants) give different F_V/F_M ratios, and this ratio sometimes differs from the value of the photochemical quantum yield obtained in the same model. While it was found that these were "errors" in Trissl's work (Falkowski *et al.* 1994, Trissl 1994; also

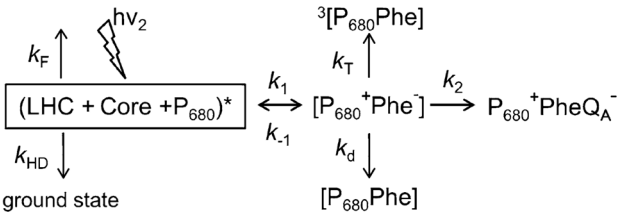

Fig. 2. A scheme for the quenching of the excited states of chlorophyll molecules with the formation of a reversible radical pair. All the pigments connected with the photosystem II (LHC + Core + P_{680}) are assumed to form a single pool, and the primary charge separation is presumed to be reversible. LHC, light-harvesting complex; the "core" represents the pigments of the core reaction center complex of PSII; P_{680} is the primary electron donor of PSII; Phe is pheophytin, the primary electron acceptor of PSII; Q_A is the primary plastoquinone (electron) acceptor of PSII; k_F is the rate constant of the radiative energy dissipation at the antenna level (fluorescence emission, as well as the delayed light emission, DLE); k_{HD} is the rate constant of nonradiative energy dissipation at the antenna level (internal conversion, quenching by triplet states, energy spillover to PSI or transfer to other PSII exogenous fluorescence quenchers); k_1 is the rate constant related to the intrinsic rate of the primary charge separation; k_{-1} is the rate constant of the radiative charge recombination that leads to re-excitation of the antenna and DLE; k_2 is the rate constant of the decay of radical pair by electron transfer to Q_A; k_T is the rate constant of the decay of the radical pair through ^{3}Chl generation; k_d is the rate constant of the decay of the radical pair through nonradiative recombination to the ground state. (Note: The rate constants k_1, k_{-1}, k_2, and k_d have different values for the open than for closed centers.) Source: Stirbet and Govindjee (2012).

see Holzwarth 1993), it is clear that the relationships between the fluorescence intensity and the processes of the deactivation of the excited state are much more complex than used in the Kitajima and Butler (1975) model which postulated irreversible photochemical reactions (*see* Lavergne and Trissl 1995). Now, below, we consider the issues related to the instruments used for fluorescence measurements.

The influence of the technical features of the fluorometer on the measured F_V/F_M values

This issue is manifested by the fact that, for the same organism and the same samples, even after identical dark adaptation, different fluorometers give different F_V/F_M values (Antal *et al.* 2009, Brown *et al.* 2019, Kromkamp and Forster 2003, Padhi *et al.* 2021). This implies that some devices give closer to the real estimates of the PSII photochemical quantum yield than others. We list below the major points related to this issue.

Determining the F_M level in the single turnover state (ST; protocols Pump-and-Probe, 100–200 µs Fast Repetition Rate) *vs*. that in the multiple turnover state (MT; protocols PAM, 100–2,000 ms Fast Repetition Rate, Fast Induction)

Single turnover state (ST) corresponds to a system with closed RCs, in which, due to the high intensity and

the short duration of the saturating flash, complete reduction of Q_A (single charge separation event) is achieved, but there is no time for the reduction of Q_B and the plastoquinone pool (PQ-pool) to take place. However, we note that the Q_B reduction can be detected after a ST flash (in particular, in Pump-and-Probe fluorometers), but this depends on the time after the ST flash, and on how long time the detection system is working. Multiple turnover state (MT) of closed RCs appears after a long-term exposure of the sample by a saturating light, when in addition to the reduction of Q_A, the plastoquinone Q_B and the PQ-pool are also reduced (during multiple charge separation events). In our opinion, besides the very large differences between the light intensities and the illumination times, used in these two types of measurements, the above is the most significant difference between the results obtained with these two methods, used for measuring F_V/F_M. Research on this topic (Samson and Bruce 1996, Kromkamp and Forster 2003, Prášil *et al.* 2018, Brown *et al.* 2019) has shown a systematic difference of 1.5–2 times in terms of F_V/F_M measured by single turnover (lower F_V/F_M) than by multiple turnover (higher F_V/F_M) methods. On the other hand, we note that the quantum efficiency of PSII (Φ_{Po}) measured by Roelofs *et al.* (1992) and Wientjes *et al.* (2013), by using other methods than Chl fluorescence induction (*see* Introduction), had values of 0.9 and 0.91, which are very close to 0.88 reported by Pfündel (1998) for the F_V/F_M ratio of 0.83 measured using MT light, and then corrected for the PSI contribution. Moreover, for the saturating single turnover flashes (< 300 ps), the ratio F_M/F_O decreases much more with the duration of the flash (France *et al.* 1992; *see* a discussion of this paper in Lazár 1999), which shows corresponding decreases in the apparent F_V/F_M values (as $F_V/F_M = 1 - 1/(F_M/F_O)$, suggesting fluorescence quenching due to the high light intensities used in single turnover measurements (*see* below for a discussion about a special quenching induced by excessive light intensities HIQ; Schreiber *et al.* 2019, Schreiber 2024).

Differences in the spectrum of the measuring exciting light source and the sensitivity spectrum of the detector

The spectrum of the measuring exciting light source and the sensitivity spectrum of the detector have a different proportion of PSI fluorescence included in the total signal, but it is only PSII fluorescence that is needed for our analysis. The optimal combination, to be used for this purpose, is blue (430–480 nm) exciting light and a narrow band-pass filter in front of the detector – tuned to the fluorescence maximum of the PSII at 680–690 nm, which cuts off the PSI fluorescence (710–800 nm) as well as a significant part of phycobilin fluorescence (< 660 nm) in cyanobacteria (Franck *et al.* 2002, Simis *et al.* 2012, Santabarbara *et al.* 2019).

On the intensity of the exciting saturating light

The use of too low as well as too high exciting light intensity is inappropriate (*see e.g.*, the theoretical results on the dependence of F_V/F_M on the light intensities used by Lazár 2003). Indeed: (i) under low photon flux density it is impossible to achieve complete closure of the RCs; as a result, the true F_M value is not reached; (ii) on the other hand, too high photon flux density results in a high rate of signal rise in the OJ phase of the Chl *a* fluorescence induction curve, making it difficult to monitor the true value of the "O" level, and, in addition, it can also induce photoinhibition; further, the first reliable point (from the sample) on the recorded fluorescence induction curve is overestimated relative to the true F_O level (*see e.g.*, Padhi *et al.* 2021); (iii) also, the too rapid arrival of photons on the PSII reaction center leads to the following problem: the relatively lowered rate of electron transfer from the oxygen-evolving complex leads to the accumulation of P_{680}^+ – a strong quencher of excited Chl states (Shinkarev and Govindjee 1993); this may also affect the F_M (*i.e.*, the P level); and, (iv) excessive high saturating MT light pulses (SP) used sometimes in PAM fluorometers, which induce the quenching of the measured F_M (called high-intensity quenching, HIQ; Schreiber *et al.* 2019), which is due to carotenoid triplet quenching *via* a singlet–triplet annihilation mechanism (*see e.g.*, Gruber *et al.* 2015). The HIQ increases linearly with the intensity of the SP pulse, but it relaxes rapidly (100–400 μs) after the pulse. The application of the maximum MT intensity provided by the MC-PAM induces a HIQ that lowers the F_M' by ~ 8%, which will lower the calculated value of the effective PSII quantum yield (Φ_{PSII}), as studied by Schreiber *et al.* (2019). Moreover, we note that the HIQ is stimulated by anoxic conditions, is not affected by DCMU treatment, and when the SP pulses are not excessive, the energy-related NPQ can significantly prevent the fluorescence decrease by HIQ.

Possible changes in the excited chlorophyll deactivation constants between the F_O and the F_M states

Returning to the initial formal model of Kitajima and Butler (1975), we emphasize that the F_V/F_M ratio will be strictly equal to the maximum photochemical quantum yield of the PSII only if the rate constants k_F, k_Q, and k_D are the same at the F_O and the F_M states, and there is no additional fluorescence quenching. The point we want to stress here is that, in fact, this may not be the case (*see e.g.*, Dau and Sauer 1992, Shinkarev and Govindjee 1993, Vredenberg and Bulychev 2003). The first extensive review of all the "players" affecting the O-J-I-P Chl fluorescence rise was published by Lazár (2006). Also, a list of the different nonphotochemical quenchers affecting the O-J-I-P transient is available in Stirbet and Govindjee (2012).

Further, it was suggested that at the F_M – determined with multiple turnover light – the k_D may have a lower value than at F_O, due to change(s) in the redox-state of the PQ-pool. In experiments with exogenous quinones and broken chloroplasts, it has been shown (*see e.g.*, Vernotte *et al.* 1979, Bukhov *et al.* 2003) that in the oxidized state, these quinones quench the fluorescence of light-harvesting complexes by a static mechanism. With the gradual

reduction of quinones, this type of quenching disappears (*see e.g.*, Vasil'ev *et al.* 1998). The effect of the oxidized PQ pool quenching was also considered in the numerical simulations of the O-J-I-P Chl fluorescence transient by Stirbet *et al*. (1998) and Lazár (2003). However, Tóth *et al.* (2005) showed that the oxidized PQ-pool does not quench Chl fluorescence in leaves (thus, in intact chloroplasts), but this possibility cannot be ruled out in advance for other systems.

Furthermore, the deactivation constants (*e.g.*, k_d shown in Fig. 2 – nonradiative recombination of transiently generated radical pairs) can change during the process of photo-conformational transitions that occur in the structure of the RC proteins during electron transfer inside it (Sipka *et al*. 2021). The specific direction, as well as how much these quenching constants affect the F_O and the F_M states, have not yet been estimated, but it is a well-known fact that conformational changes do occur in the RCs (*e.g.*, Kleinfeld *et al.* 1984, Nagy *et al.* 2008). It is, thus, obvious that such conformational changes can affect the properties of Chl molecules embedded in the protein matrix of the RC since they are known to interact with the polar groups of proteins as well as with other chromophores (Moise and Moya 2004, Schansker *et al*. 2011). For further information on the issues related to the different levels of nonphotochemical quenching (NPQ) of Chl *a* fluorescence during the recording of fluorescence induction, *see* Garab (2024); also *see* Schreiber (2024) for different opinions on this topic. In addition, we refer the readers to Belyaeva *et al.* (2015), Riznichenko *et al.* (2022a), and Riznichenko *et al.* (2022b), where the above aspects have been studied for Chl fluorescence transients induced after a saturating 10-ns ST flash, by using mathematical simulation models (*i.e.*, with real fluorescence signal fitting).

The influence of the taxonomic affiliation of the sample, its physiological state, and the preparation methods on the measurement of F_V/F_M

The photosynthetic apparatus of cyanobacteria, prokaryotes, after dark adaptation, is usually in the State 2 (absorbed light energy is redistributed in favor of PSI), and this is significantly different from that in eukaryotic microalgae and in higher plants, which, when dark-adapted, are in State 1 where the absorbed light energy is distributed in favor of PSII (Stirbet *et al.* 2019, Calzadilla and Kirilovsky 2020). In State 2, there is an increased contribution of PSI fluorescence. Further, the reduction of the PQ-pool by respiration in cyanobacteria leads to an increase in the initial level of fluorescence (F_O). Here, the measured F_O also includes some phycobilin fluorescence from the phycobilisomes, unless care is taken to correct for it. As a result, the apparent value of F_V/F_M (after dark adaptation) in cyanobacteria rarely exceeds 0.5, but in the eukaryotes, it is between 0.7 and 0.83 (Campbell *et al.* 1998, Simis *et al.* 2012). However, adaptation of cyanobacteria by exposing them to far-red light (> 700 nm) or blue light (400–480 nm) leads them to be in State 1, where the F_V/F_M value becomes high (0.6–0.65; *see e.g.*, Mullineaux and Allen 1990, Voloshina *et al*. 2016).

A partial reduction of the PQ-pool in the dark is also known to occur in the eukaryotes, which is due to the activity of chlororespiration (Bennoun 2002, Miloslavina *et al*. 2007). In this case, the value of F_O has been shown to increase, while the dark-adapted F_M is slightly quenched; thus, the F_V/F_M value is underestimated (Feild *et al*. 1998).

During the process of photoacclimation, and when photosynthetic systems are under stress conditions, a sustained nonphotochemical quenching can occur, which is a quenching of the Chl excited state that persists for a long time (Schindler and Lichtenthaler 1996). A specific mechanism of this quenching is an increase in the degree of de-epoxidation of the xanthophyll cycle carotenoids (accumulation of zeaxanthin or diatoxanthin) and an increase in the total content of xanthophyll carotenoids in algae, as well as in plants (Horton 2012, Ruban and Wilson 2021). Another example of nonphotochemical quenching of Chl *a* fluorescence, known from measurements under natural conditions, especially during midday under high light intensity, is photoinhibition (Murata *et al*. 2007). Here, the quenching of the excited states is caused by the photooxidation of the D1 protein, located in PSII; further, the F_V/F_M values decrease because of a much more pronounced decrease in the F_M than that in the F_O (Ohad *et al.* 1990).

Additional aspects of the practical use of short and long saturating flashes

In some experiments, measuring F_M with a short saturating flash of 0.1–1 ms duration is more preferable compared to measuring it with longer (500–1,000 ms) saturating flashes. Let us consider two examples. The first example is repeated measurements of F_V/F_M on the same cells or leaf areas. Such measurements can be carried out on the following systems:

- In long-term experiments on a leaf, lichens, and algal thalli – under artificial or natural light illumination conditions, and with parallel measurement of gas-exchange processes;
- On natural phytoplankton samples, at low Chl content, fluorescence signals have a low signal-to-noise ratio that results in random errors in estimating the Φ_{Po} values; to increase accuracy, repeated measurements of F_V/F_M and averaging must be applied; and,
- On immobilized single cells to study their life cycle.

In our experience, a single long (200–500 ms) saturating flash applied every 20 min (or more often) significantly affects several physiological processes in cells. This is evident from the data on the inhibited cell growth rate (Fig. 3*A*,*C*) and the gradual decrease in PSII photochemical quantum yield (Fig. 3*B*). On the contrary, the use of short (0.1–1 ms) saturating flashes allows one to measure F_V/F_M much more frequently. Further, it is also useful for monitoring rapid cell growth, cell cycle stages, or cell responses to the effects of external factors (such as – toxins, gases, and mineral nutrients).

A still another case concerns F_V/F_M measurements under conditions when the samples are moved at a high

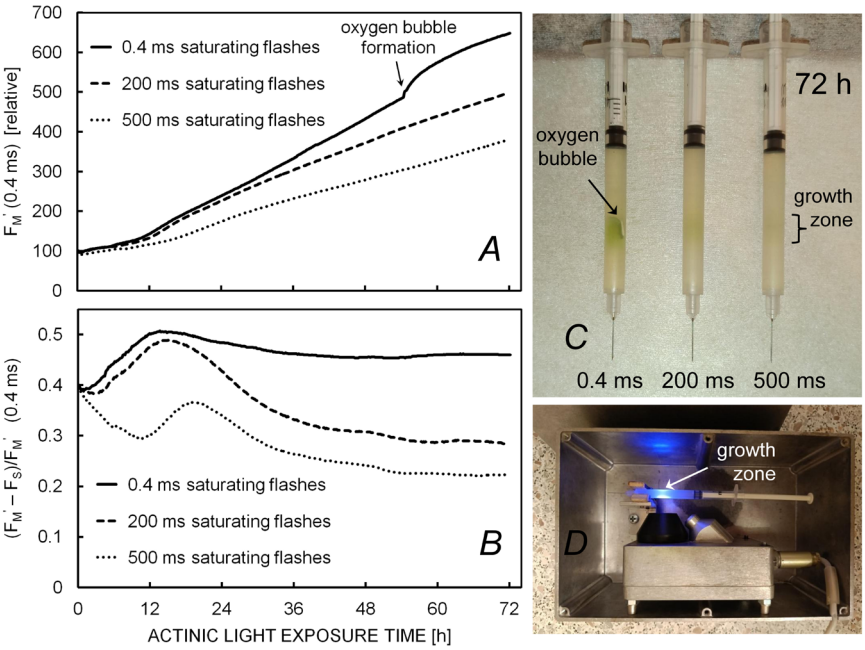

Fig. 3. Effect of short and long repeated saturating light flashes on the growth of immobilized cells of *Chlorella vulgaris* Beijer. (I. Konyukhov, unpublished results). The cells were suspended at +40°C in liquid 1% agar on Tamiya 1:30 nutrient medium with 1 mg cm^{-3} of baking soda added as a carbon source. After the samples were cooled to room temperature and the gel formed, the cells were immobilized. The cells in the closed box were kept at +25°C, for 72 h under blue (450 nm) actinic light [35 µmol(photons) m^{-2} s^{-1}], obtained from a Fast Repetition Rate fluorometer, shown in the insert *D*. Every 10 min the fluorometer generated saturating 450-nm flashes [8,000 µmol(photons) m^{-2} s^{-1}] to measure F_M' and to obtain the effective photochemical quantum yield of PSII [$(F_M' - F_S)/F_M'$ ratio]. Three series of experiments were conducted, each with a different duration of the saturating flash: 0.4 ms, 200 ms, and 500 ms. *A* – kinetics of cell culture growth based on fluorescence intensity (F_M' measured 0.4 ms after the start of the saturating flash sequence). *B* – changes in the effective photochemical yield of PSII in the same immobilized samples. *C* – photographs of samples with algal cells at the end of a 72-h light incubation period. *D* – a fluorometer and a fixed sample inside a light-proof box with the lid removed. (We note that the long light flashes caused inhibitory effects on cell growth rate and PSII activity.)

speed in front of the fluorescence detector as shown in Fig. 4 for F_V/F_M measurements performed through the transparent wall of a photobioreactor. This situation is also relevant in the case of vertical water column probing by means of submersible fluorometers in marine research or in cases when variable Chl *a* fluorescence is measured on photosynthetic samples in flowing water: in a flow cell or in rivers and streams. In these conditions – under an MT saturating flash – some cells can "escape" from the light illumination beam. However, during the "saturation" process (due to high light), they may get replaced (or not replaced) by new cells with open RCs. Thus, the F_V/F_M values will be systematically underestimated, and the result will depend on the mixing rate (Fig. 4*B*, the "steps" on the red F_V/F_M curve when the stirrer is turned on and when its speed is increased or decreased).

In addition, our results on the F_V/F_M measurements under long MT flashes show that the system is very sensitive to the presence of large bubbles. For Fig. 4*A*,*B*, generation of large bubbles was induced within the time interval between 0.7 and 1.2 h of cultivation. These bubbles could "push out" the cells from the photometric zone in front of the fluorometer's detector. Thus, the measured F_O and the F_M values on the OJIP curve may correspond to those from different cells and even from different numbers of cells (Fig. 4*C*; *see* the three OJIP curves – with dotted lines). Therefore, such measurements sometimes give unrealistically high values of F_V/F_M: 0.9 or more (Fig. 4*B*; *see* the red curve, for samples within 0.7–1.2 h period). Using shorter saturating flashes, in the millisecond and sub-millisecond range, lower average F_V/F_M values are obtained, and they are much more stable in the presence of bubbles and do not depend on the mixing rate (*cf.* blue *vs.* red curve in Fig. 4*B*).

Concluding remarks

Despite the above complications and theoretical considerations, the Kitajima and Butler model of 1975 is widely used in the analysis of experimental fluorescence induction data since the reliability of the fluorescence parameters F_V/F_M (*i.e.*, the maximum efficiency of PSII photochemistry) and the closely related parameter Φ_{PSII} (*i.e.*, the effective quantum yield of PSII in light) has been verified in many experimental studies (*see* the Introduction).

But, as discussed earlier in this paper, when the PSII RCs are not completely open or closed, respectively, or, when the sample is in State 2 after darkness, and when the PSI fluorescence (or that of other pigments) have a significant contribution to the measured fluorescence, the calculated F_V/F_M ratios do not express the real

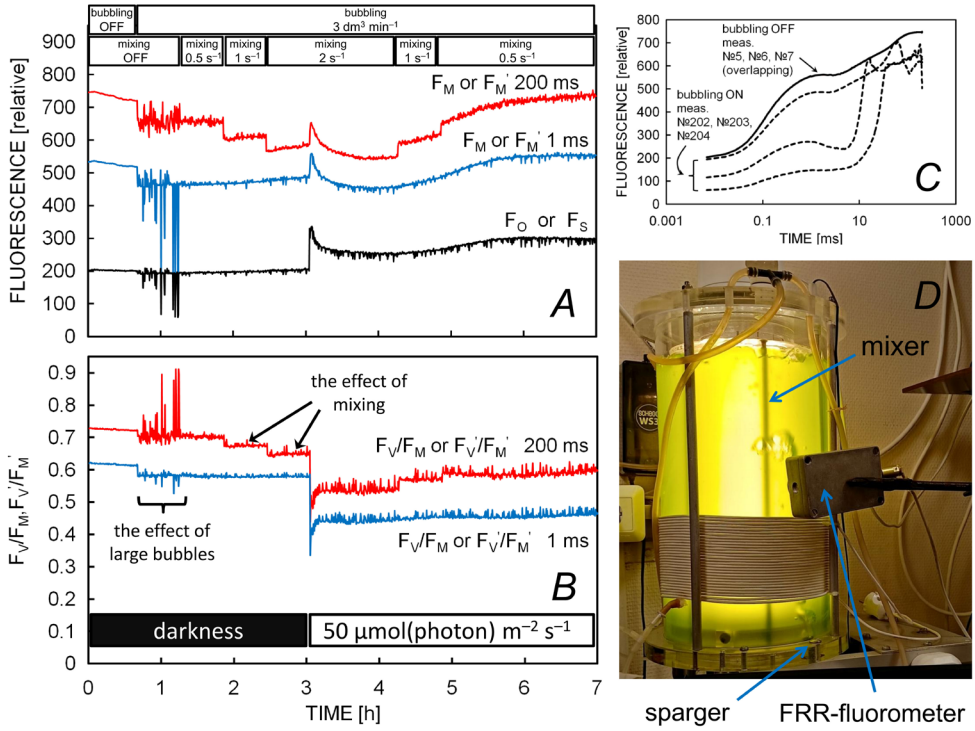

Fig. 4. The effects of stirring and bubbling on long-term repeated measurements of F_V/F_M in a photobioreactor on *Chlorella vulgaris* Beijer. (I. Konyukhov, unpublished results). In the 5-dm³ photobioreactor, shown in the insert *D*, the values of F_V'/F_M' were quantified every 20 seconds by means of an FRR-fluorometer directly through the transparent vessel wall [saturating 450-nm light – 8,000 µmol(photons) m^{-2} s^{-1}]. Mixing and bubbling were controlled independently. F_V'/F_M' values were calculated according to two formulas: one for F_M' determined at 1-ms saturation flash, and another for F_M' determined at 200-ms saturation flash. During the first three hours of the experiment, the samples were in the dark. *A* – changes in initial (F_O or F_S) and maximum fluorescence (F_M or F_M') over time in response to changes in stirring and bubbling conditions (changes in bubbling and stirring rates are shown at the top). *B* – changes in the F_V/F_M ratio in the same experiment. The moment of turning on the photobioreactor light is shown on the x-axis. *C* – three consecutive measurements of the OJIP curve without bubbling and three consecutive measurements with bubbling. *D* – an experimental photobioreactor (bubbling on, mixing off).

maximum Φ_{Po} of the sample, but smaller "apparent Φ_{Po}". To make the F_V/F_M ratio as close as possible to the actual value of Φ_{Po}, the following practical measures must be taken into account:

• Minimize the contribution of PSI fluorescence (use: optimal excitation/emission spectral bands and pre-stimulation of State 2 to State 1 transition in the case of cyanobacteria).

• For the algae, eliminate the possible increase of F_O in the dark due to chlororespiration induced during too long a dark adaptation.

• Ensure the use of high intensity (saturating) light to close the RCs, but not excessive (*see* our earlier discussion about HIQ, Schreiber *et al*. 2019).

We also note that, while there are large differences between the F_V/F_M^{ST} values measured with Fast Repetition Rate (FRR) fluorometers, and the F_V/F_M^{MT} values measured with the Pulse-Amplitude Modulation (PAM) fluorometer and the continuous-excitation fluorometers, the Fast Repetition Rate (FRR) fluorometers are also providing a strong 100-ms MT pulse, which is used for measurements in light-adapted conditions, by emulating the PAM method (*see* Fig. 1 in Kolber *et al*. 1998; *cf*. Gorbunov and Falkowski 2022).

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Appendix. Derivation of the equation (8).

We introduce the following time-dependent variables: [Chl*] – the relative number of excited chlorophyll molecules in the sample (from 0 to 1); [Chl] – the relative number of deactivated chlorophyll molecules in the sample (from 0 to 1); $V_{0\text{-}1}$ – number of S_0–S_1 transitions per time unit; $V_{1\text{-}0}$ – number of S_1–S_0 transitions per time unit.

A total number of chlorophyll molecules C_o is assumed to be stable in time; thus, the following conservation law can be written:

$$[\text{Chl}] + [\text{Chl}^*] = C_o = 1 \qquad (10)$$

$C_o = 1$ as we use normalized values of [Chl] and [Chl*] on the total number of chlorophyll molecules.

S_0–S_1 transitions occur when (i) excitation light is applied and (ii) when this light is absorbed by a chlorophyll molecule being in the ground state. For a light-limited process we can write:

$$V_{0\text{-}1} = [\text{Chl}] \times \text{PFD} \times \sigma \times N_A \tag{11}$$

where PFD is a photon flux density used to excite fluorescence [mol(photons) m^{-2} s^{-1}]; σ is an effective absorption cross section of a chlorophyll molecule [m^2]; N_A – Avogadro constant, 6×10^{23} mol^{-1}.

S_1–S_0 transitions occur spontaneously in excited molecules, and their rate ($V_{1\text{-}0}$) is proportional to the number of excited molecules [Chl*]. As it was postulated by Kitajima and Butler (1975), the S_1–S_0 transition is sum of independent first order reactions. So, using the same designations as used in equations (1)–(3) we can write:

$$V_{1\text{-}0} = [\text{Chl*}] \times k_{\text{total}} \tag{12}$$

where $k_{\text{total}} = k_F + k_D + k_Q + k_P$.

The $V_{0\text{-}1}$ process results in accumulation of Chl* molecules, whereas $V_{1\text{-}0}$ process acts in the opposite direction. Taking this into account we have the following differential equation:

$$d[\text{Chl*}]/dt = V_{0\text{-}1} - V_{1\text{-}0} \tag{13}$$

Using equations (11), (12), and (13) the above can be rewritten as:

$$d[\text{Chl*}]/dt = (1 - [\text{Chl*}]) \times \text{PFD} \times \sigma \times N_A - [\text{Chl*}] \times k_{\text{total}} \tag{14}$$

This equation can be solved on the time interval after instant switching on the excitation light, using the following initial condition: [Chl*] = 0 at t = 0. The result is:

$$[\text{Chl*}] = \frac{\text{PFD} \times \sigma \times N_A}{\text{PFD} \times \sigma \times N_A + k_{\text{total}}} \left(1 - e^{-(\text{PFD} \times \sigma \times N_A + k_{\text{total}}) \times t}\right) \tag{15}$$

The fluorescence intensity, F(t), is simply the product of [Chl*] and fluorescence rate constant k_F:

$$F(t) = \frac{k_F \times \text{PFD} \times \sigma \times N_A}{\text{PFD} \times \sigma \times N_A + k_{\text{total}}} \left(1 - e^{-(\text{PFD} \times \sigma \times N_A + k_{\text{total}}) \times t}\right) \tag{16}$$

And after replacement of some of the symbols:

$$F(t) = F_O \times \left(1 - e^{-\frac{t}{\tau}}\right)$$

where

$$F_o = \frac{k_F \times \text{PFD} \times \sigma \times N_A}{\text{PFD} \times \sigma \times N_A + k_{\text{total}}}$$

and

$$\tau = \frac{1}{\text{PFD} \times \sigma \times N_A + k_{\text{total}}} \tag{17}$$