The effect of light pulse intervals on PAM measurements in various microalgae

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Introduction

Pulse Amplitude Modulation technique (PAM) is a commonly used noninvasive method to study photosynthetic activity through chlorophyll fluorescence measurements. Here we provide insight into an ongoing project where we test the hypothesis that the time intervals between the pulses during the measurements using PAM might affect the variable fluorescence and therefore a standardised protocol is needed. Moreover, we investigate the possible effect on the bigger scale growing *C. reinhardtii* in a flat panel photobioreactor. To allow for a PAM measurements to be taken in the reactor we build our own flash panel.

Planning experiments using mathematical models

Precisely calibrated mathematical models allow for a quick, low-cost scan through a wide range of possible experimental settings and help to identify those that looks most promising. Thus, mathematical models can become a valuable resource for experimentalists.

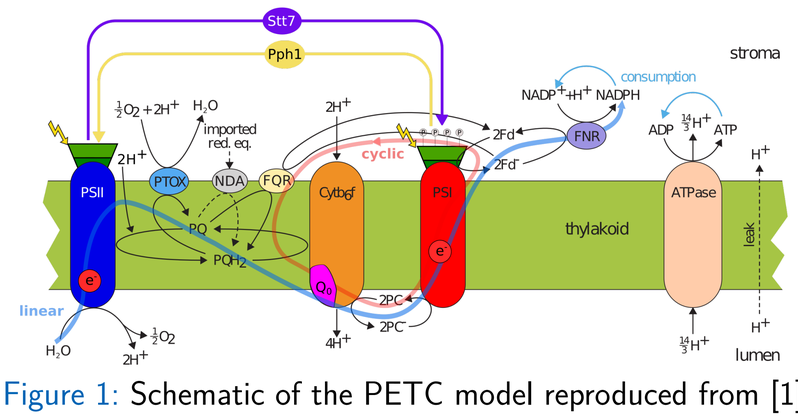

Figure 1: Schematic of the PETC model reproduced from [1]

Here we use a mathematical model of the photosynthetic electron transport chain [1] to simulate PAM measurements with various time intervals at which the pulses of light are applied.

Simulation of PAM curves

PAM protocols were simulated with a dark-light-dark protocol for a time interval of 60 and 240 seconds between the pulses of saturating light. The plots shown in in figure 2 show the fluorescence trace and the state of the plastiquinone pool (PQ).
The fluorescence trace shows faster and stronger relaxation in the dark if the pulse interval is greater. During the moderate light phase the fluorescence traces differ only slightly but the PQ pool gets less reduced for fewer flashes. This suggests that a high density of saturating light pulses put additional stress on the organisms in question and therefore might interfere with the measurement of photosynthetic parameters.

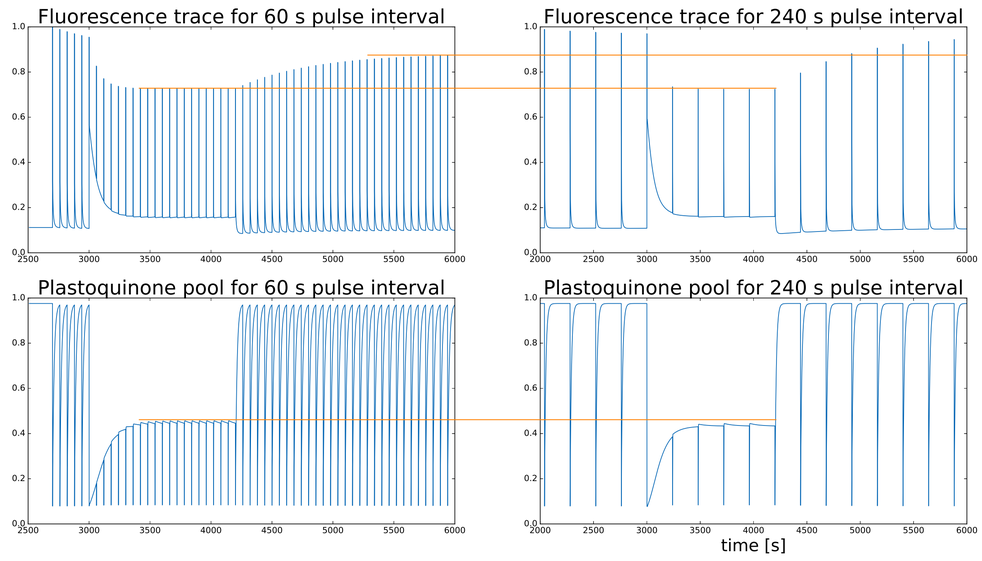

Figure 2: Fluorescence trace (top) and state of the PQ pool (bottom) of *in silico* PAM simulations

Building the light panel

We used FMT150 flat panel photobioreactors (PSI, CZ). Most bioreactors are not designed to perform sophisticated light experiments such as PAM, nor allow for novel set-ups. The strongest available light panel had an output of 1500 $\mu mol\ m^{-2}\ s^{-1}$ and thus was not sufficient to provide the saturating pulses of light required by the PAM method. This issue was tackled by building a prototype of a custom light panel using 10x 10 watts LEDs (see figure 3b, 3c) powered by a common ATX PC power supply and controlled by a Raspberry Pi 2 single-board computer. The spectra of our prototype (see figure 3d) was measured using an Avantes radiospectrometer (Avantes, NL).

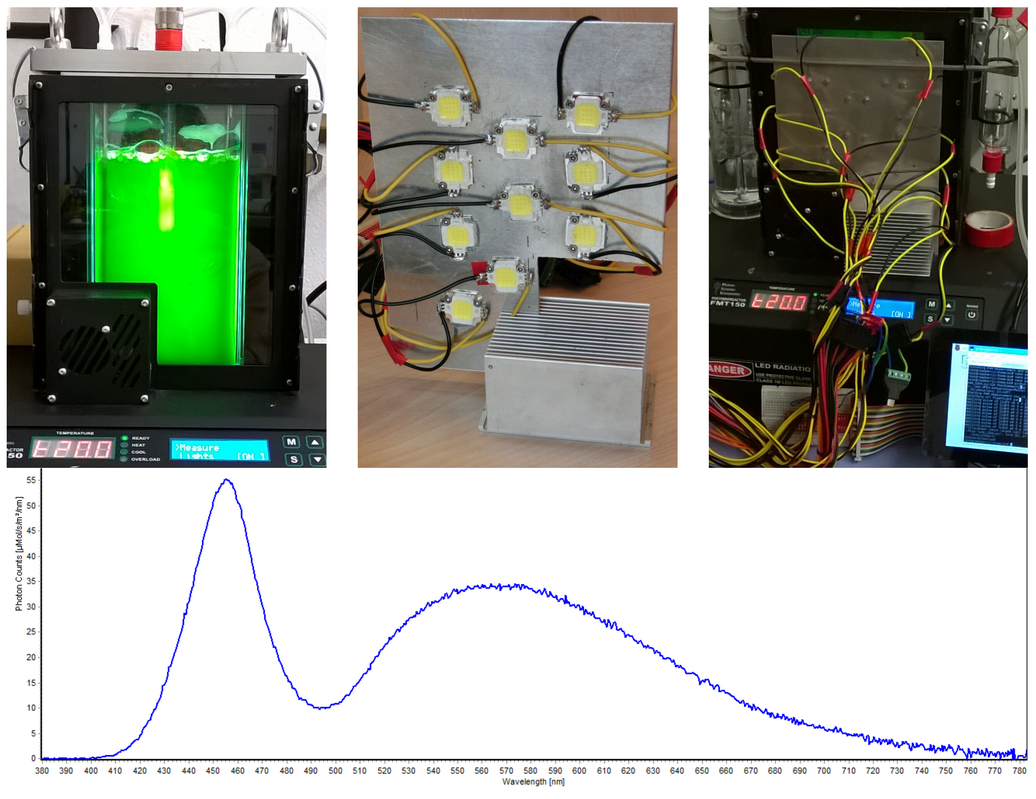

Figure 3: Photobioreactor, custom build light panel and spectra

Time resolution is a limited factor in bioreactors

Figure 4 shows the fluorescence trace of *C. reinhardtii* grown in a PBR using the same light protocol that was used for the *in silico* simulation. In an ideal setting the fluorescence trace would be measured immediately prior and after each pulse of light with the pulse duration as short as possible. Here, we were limited by the time resolution of the PBR main device and controlling software which is limited to *ca.* one second and of quite low accuracy resulting in rather unreliable fluorescence trace curves.

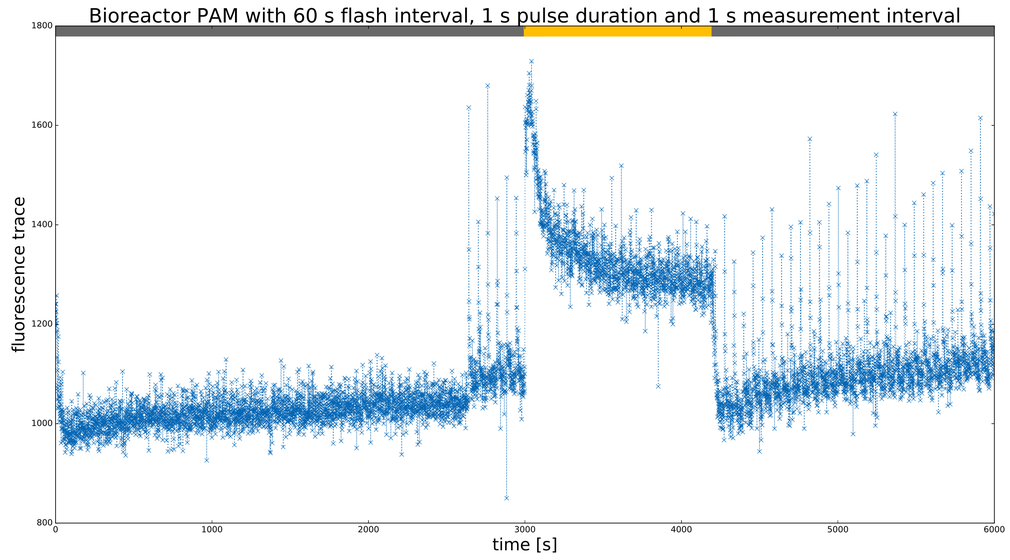

Figure 4: PAM with synchronization problems

Summary and outlook

Currently we are in the process of adapting the model to various other micro-algae like diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. We are also improving our technical set-up to allow for more precise PAM measurements on a whole culture growing in a photobioreactor.

[1] Ebenhöh, O. *et. al.*, *Philos. Trans. R. Soc. Lond., B, Biol. Sci.*

See also: Poster by Simon Schliesky *et. al.* YAS | ENCAPP 2016 poster No. 35 | 24