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High-resolution photosynthesis-irradiance curves in microalgae

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Reviewer 2: Lijin Tian

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Manuscript reviewed 2022-08-07: *Only major points included.*

Reviewer 2

Could the PI curve be affected by the initial level of oxygen in the solution? If yes, please clarify how the pre-treatments of cells should be done in this regard.

Authors

We started the experiments after equilibration of the measuring chamber with atmospheric oxygen, which corresponded to a concentration of 220 µM. Since all experiments were started with the same initial oxygen levels, this should not have influenced the PI curves (we clarified this point in the caption of Figure 1). Moreover, in the experiments where elevated oxygen concentrations were reached, we avoided saturation of the sensor by opening the chamber and equilibrating the sample for few seconds with air, as suggested by the manufacturer (a reference for this: https://doi.org/10.26124/mitofit:2021-0005).

Reviewer 2

According to the authors, the red plot in Fig 1A is the time-derivative of oxygen concentration line in blue, however, the blue line seems to me pretty smooth, I don't see any turning point that connects to the sharp increases in the red line. Was the change so small?

Authors

The red plot (O_2 flux) of Figure 1A is, indeed, the time-derivative of the blue plot (O_2 concentration). The changes in the slope of the blue plot are subtle, because we are increasing the light intensity little by little, but the resolution of the system enables a reliable quantification of these changes, since also the signal-to-noise is very high.

Reviewer 2

When talking about unprecedented accuracy, would be nicer to make a quantitative comparison with other electrodes, please do so only if it is possible.

Authors

We did not perform a systematic comparison between other electrodes (e.g. Hansatech oxygraph) and the NextGen-O2k because it was not the scope of this work. Also, we never performed PI curves in the past using the Hansatech device because we could not control the light intensity as finely as we can do with the PhotoBiology module here employed. However, we agree with the reviewer about the importance of discussing this point in our manuscript. Therefore, in the revised version of our manuscript (lines 231-232) we added a reference to a previous publication of ours where we used a Hansatech device with Nannochloropsis, which required a concentration higher than 300 x10^6 cells mL-1, i.e. more than 30 times than the working concentration in the current work.

Reviewer 2

Please briefly explain how this type of Fluororespirometer works, and which wavelength it employs. And why blue LED was used in its PB module?

Authors

In our manuscript, we did not explain in detail the principles behind the functionality of the device because they are the same of any Clark-type electrode, which we assume the readers can easily retrieve in literature. The PhotoBiology module consists of a light source that is directly controlled by the NextGen-O2k. As we described in the Methods section, the LED source in our case is blue (range of emitting wavelength 439-457 nm with a peak at 451 nm). We used blue light as this was the only LED available for the PB module.