CHLOROPHYLL FLUORESCENCE INSTRUMENTATION FOR A RAPID, *IN SITU* MEASUREMENT OF ALGAL DENSITY

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Abstract

In the project reported, we are developing an instrument for measuring algal density based on the detection of chlorophyll fluorescence. Following the adjustment of several parameters defined during preliminary analyses, measurements were made on different concentrations of model green and blue algal cultures. Fluorescent signal intensities measured by the prototypes of the fluorometer module were compared to values determined by other, widely used methods for estimation of algal density (i.e. Bürker chamber cell counting, optical density measurement and chlorophyll-*a* measurement with ethanol extraction method). Fluorometer results correlated well with the other methods, resulting high correlation coefficients (R^2>0.9%). Limits of detection and limits of quantification showed a decreasing trend during the development phases resulting in a highly sensitive instrument.

Introduction

A common problem affecting surface waters is the load of organic materials and plant nutrients by human activity. This leads to eutrophication and the excessive growth of algae. This influences the aquatic ecosystem of water bodies, their ecological status, and alters water use opportunities. Algae are good indicators of water quality; therefore, their study is an important element in water monitoring. Several methods exist to estimate their abundance, one of which is the measurement of chlorophyll-*a*, the main photosynthetic pigment in algae. Algal species contain various types of pigment, the diversified detection of which on the basis of different light spectra used in the process can be used to estimate algal composition.

The phenomenon of fluorescence can be used to measure chlorophyll, during which the sample is excited by electromagnetic radiation at a given wavelength and in response, it emits light at another, somewhat higher wavelength. The method of fluorescence-based chlorophyll measurement was developed during the early 1970s [1] and several attempts were made later to separate the main algal groups from each other based on their different pigment composition [2, 3]. Fluorescence-based instruments for estimating chlorophyll content and algal composition are available today [4], although their effectiveness is often disputed [5].

Within the framework of a consortium of Project Aquafluosense (NVKP_16-1-2016-0049) [6], we develop a complex, modular instrument for water quality measurements. Our current goal reported here is to develop a module for measuring algal density and composition based on fluorescence, operating with higher sensitivity and efficiency than previous instruments.

Experimental

During the module development, several instrument prototypes were tested with specific characteristics presented in Table 1.

Table 1. Fluorescence measurement instrument prototypes at developmental phases

Instrument characteristics	FluoroMeter Modul (FMM)	Chlorophyll Fluorometer (CFM4Ch)	Fluorometer with dichroic system (FDS)
Excitation source	Laser diode (10 mW)	Laser diode (>25 mW)	LED-light source
Excitation wavelength (nm)	635	637	470, 630
Detection wavelength (nm)	690 (Δλ=10) 735 (Δλ=10)	690 (Δλ=10) 735 (Δλ=10) 716 (Δλ=43) 708 (Δλ=75)	716 (Δλ=43) 708 (Δλ=75)
Number of channels	2	4	2

In order to select optimal excitation wavelengths, the emission fluorescence spectra of species of different algal phyla were recorded at excitation wavelengths of 420, 470, and 630 nm. The test species belonging to the blue-green algae (Cyanophyta) were *Cylindrospermopsis raciborskii* (CR) and *Microcystis aeruginosa* (MA), and test species of green algae (Chlorophyta) were *Desmodesmus subspicatus* (DS), *Pseudokirchneriella subcapitata* (PS) and *Scenedesmus obtusiusculus* (SO). The basis of the separation is that while the photosynthetic system of blue-greens contains only chlorophyll-*a*, green algae additionally contain chlorophyll-*b*. The excitation wavelengths 470 nm and 630 nm were selected for the latest fluorometer prototype. At these excitation wavelengths, emission peaks were detected at 690 and 660 nm, respectively. The detection wavelengths were selected taking into account the emission spectra, as well as other important electrotechnical and optical aspects required by the module development (Table 1.).

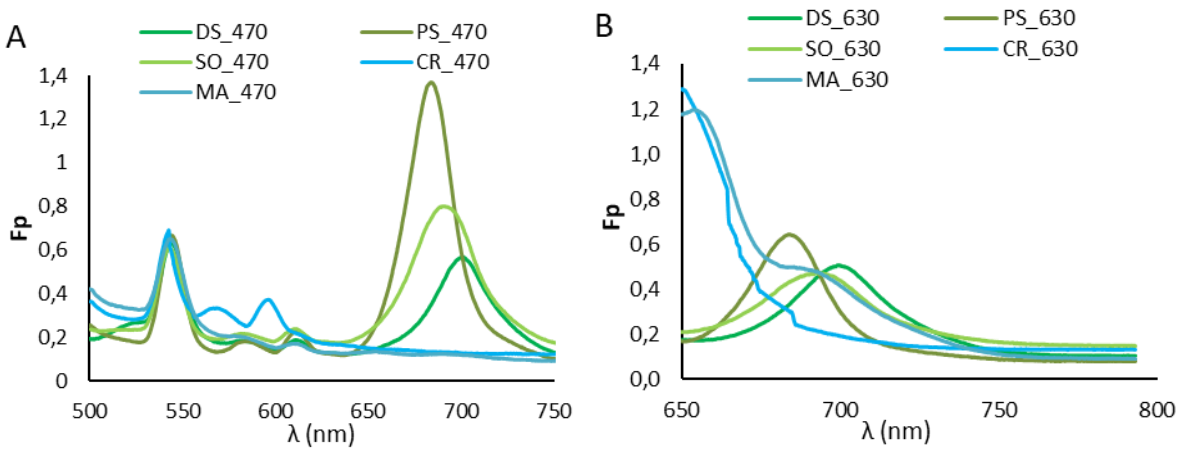

Figure 1. Emission spectra of different algal species at excitation wavelengths of 470 nm (A) and 630 nm (B).

Preliminary experiments were performed to examine and set additional parameters of the module. During these measurements, reflection was measured on microplates of different colors, using a continuous increase of light intensity, in order to find the microplate producing a stronger signal. Additionally, the effect of dark adaptation on the fluorescent signal was studied, during which a period of 10 minutes of dark incubation of a dilution series of samples before their measurement was applied. The fluorescent signals were then compared to those measurements without preceding dark incubation. Reflection was studied on three algal culture media and distilled water, using black and white microplates. Dark adaptation was studied on a dilution series of algal cultures.

Using the parameters set during the preliminary experiments, the applicability of the FluoroMeter Module (FMM), the Chlorophyll Fluorometer (CFM4ch) and the Fluorometer with dichroic system (FDS) prototypes were tested on the monocultures of the green algae *Pseudokirchneriella subcapitata* and the blue-green *Microcystis aeruginosa*.

Results were validated by other methods measuring algal density. These were (i) the detection of optical density measured spectrophotometrically at 750 nm, (ii) microscopic cell counting using a Bürker-chamber and (iii) chlorophyll measurement with the organic solvent (ethanol) extraction method. Finally, the limits of detection (LODs) and limits of quantification (LOQs) of the prototypes were determined and compared.

Results and discussion

The effect of the microplate color on the fluorescence signal is shown in Figure 2. The signal did not increase with increasing light intensity over time and remained constant on the black microplate. In contrast, it showed an increasing trend on the white microplate, along light intensity over time, in all the algae culture media (Z8, Allen, Diat.) and in distilled water (DW). The reason for this is probably that the black microplate absorbs light, whereas the white plate produces a well detectable signal. Based on these results, white the microplate was selected for the subsequent analyses.

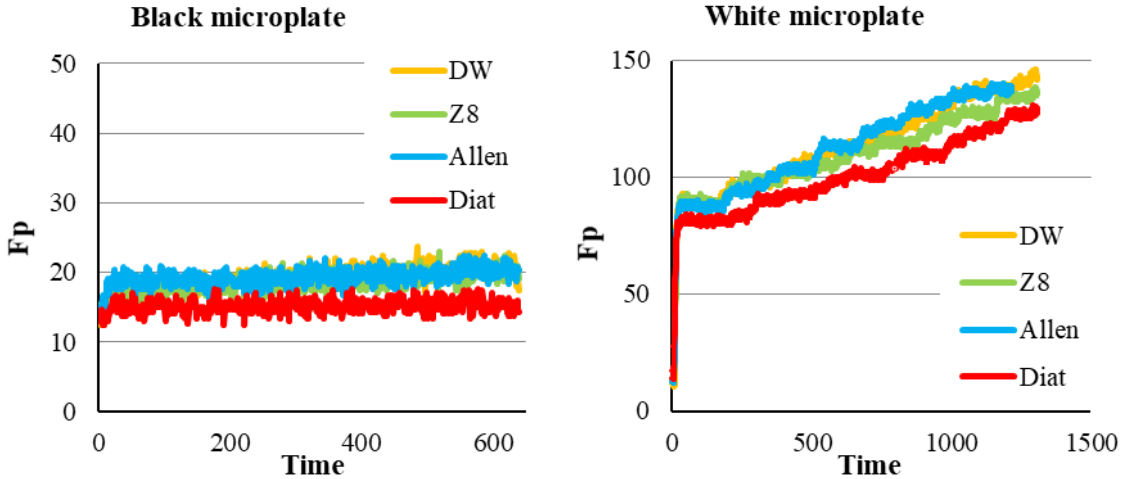

Figure 2. Reflection measured on microplates of different colors with increasing light intensity over time.

Correlation between signals of samples obtained with and without dark adaptation is presented on Figure 3. The R^2 value of the fitted line is 0.99 showing that the two datasets correlated strongly and significantly. Based on these findings, we conclude that the application of dark adaptation has no effect on the extent of the obtained fluorescence signal. Thus, in the future, it is not necessary to apply dark adaptation before our measurements.

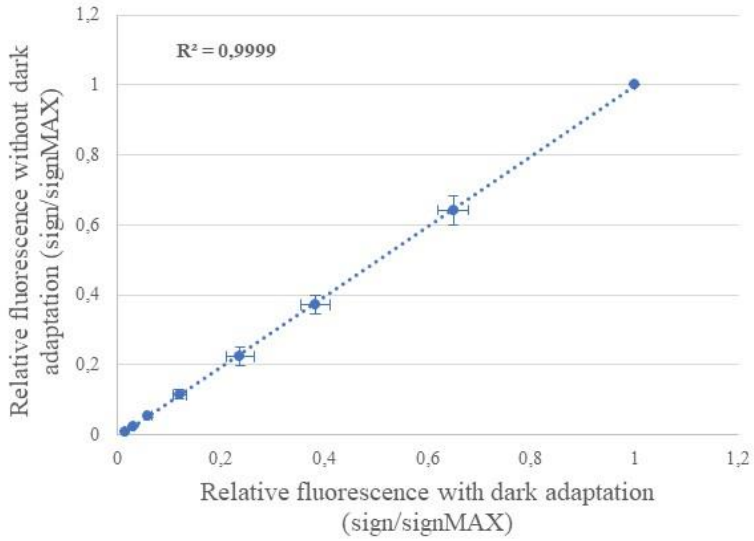

Figure 3. Effect of dark adaptation on the magnitude of the fluorescence signal.

Results obtained with the different abundance-measuring methods on the same samples were compared with the three module prototypes. The R^2 values of the fitted linear models on the correlations are presented in Table 2. Results are presented separately for the monocultures of the two algae species. In each case, we found very strong and significant correlations with R^2 coefficients >0.90, except for one case where it was 0.84.

Table 2. Correlation coefficients between densities of model green and blue algae determined by different methods.

		P. subcapitata			***M. aeruginosa***	
Prototype	**FMM**	**CFM4ch**	**FDS**	**FMM**	**CFM4ch**	**FDS**
OD	0.98	0.97 0.95	0.99 0.98	0.99	0.99 0.98	0.96 0.94
Bürker-chamber	0.98	0.95 0.98	0.94 0.98	0.99	0.99 0.99	0.95 0.92
Chl-*a* extraction	0.99	0.97 0.84	0.90 0.95	0.99	0.99 0.98	0.94 0.94

Calculated LOD and LOQ values for the three module prototypes are shown in Table 3. Based on the correlation with the cell counting method, values are interpreted in cells per milliliter, based on the *P. subcapitata* species. LOD and LOQ values showed a continuous decrease along the development phase of the module with LOD values of $4.01*10^6$, $2.22*10^3$ and $8.12*10^6$ cells/ml, and LOQ values of $8.12*10^4$, $2.65*10^5$ and $6.10*10^3$ cells/ml.

Table 3. Limit of detection and limit of quantification values for the three prototypes of the fluorometer module.

	FMM	**CFM4Ch**	**FDS**
LOD (cells/ml)	$4.01*10^6$	$2.22*10^3$	$3.70*10^3$
LOQ (cells/ml)	$8.12*10^6$	$2.65*10^5$	$6.10*10^3$

Conclusion

We conclude that the present prototype of the module gives good quality results that are validated by other methods used for the quantification of algal biomass. Both the limit of detection and limit of quantification decreased with the development process and the present prototype is considered sensitive and efficient in regard to these parameters. Our further plans for the development contain the improvement of the capacity to separate major algae groups based on their photosynthetic pigment composition, while maintaining detection and quantification limits low. We aim to enlarge its applicability to natural water samples where complex communities are present.

Acknowledgements

This research was supported by project Aquafluosense, NVKP_16-1-2016 0049 funded by the National Research, Development and Innovation Fund of Hungary within the National Competitiveness and Excellence Program.

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