Inhibition of the photosynthesis of *Coccomyxa subellipsoidea TL4* algae by diuron– comparison with the cyanobacteria *Synechococcus elongatus PCC 7942 and analytical exploitation*

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Introduction

Water pollution is a major concern due to its threat to animal's and human's health [1]. Among early warning systems that can alert about water pollution, the sensors based on the inhibition of photosynthesis of cyanobacteria, algae and plants show great potential for the detection in water of heavy metals and several classes of herbicides. widely used in agriculture [2]. Diuron, a phenylurea herbicide that persists longtime in water and can cause skin irritation, cancer, and mutations [3] was often employed as a model inhibitor of photosynthesis when developing biosensors. While cyanobacteria *Synechococcus elongatus* PCC 7942 was often chosen as biological recognition element in such biosensors, there is a continuous quest for novel phototrophs that are not only more sensitive to pollutants but can also provide stable sensors [4].

The present study compares whole cells and subcellular preparations (thylakoids and photosystem II) from the cyanobacteria *Synechococcus elongatus* PCC 7942 and the microalgae *Coccomyxa subellipsoidea* TL4, in terms of morphology and degree of photosynthesis inhibition by diuron. The stability of the various cellular and subcellular preparations was also investigated as a critical step in the development of a biosensor.

Experimental

Synechococcus elongatus PCC 7942 were from the collection of Babes Bolyai University, Cluj Napoca, Romania while *Coccomyxa subellipsoide*a TL4 microalgae were isolated from Scarisoara ice cave, Romania. Thylakoids and photosystem II preparations were obtained according to known protocols [5]. Optical microscopy and Atomic Force Microscopy (AFM) were used for imaging the whole cells and respectively the thylakoids from *Synechococcus elongatus* PCC 7942 and *Coccomyxa subellipsoide*a TL4. AFM measurements were performed with a Nanowizard II AFM instrument (from JPK, Germany) operating in intermittent contact in air. Thylakoids were diluted 20 times in 10mM Tris buffer pH 7.4, 150 mM KCl, and adsorbed onto poly(diallyldimethylammonium) (PDDA)-modifiedmica for 30 min. The modified mica was washed with buffer and water to remove loosely bond thylakoids.

Chronoamperometry was used to measure the current generated by the photosynthesis of the various biological preparations in presence and in absence of diuron. Carbon nanotubes electrodes (Metrohm Dropsens, Spain, DRP110D CNT), were polarized at +650 mV versus Ag and 2,6-dichlorobenzoquinone 0.5 mM was added in the electrolyte as an electrochemical mediator. For *Coccomyxa subellipsoide*a TL4, the assays were conducted at an applied potential of +400 mV in the presence of 0.7 mM hexacyanoferrate (II) as mediator. Cycles of 1 min light followed by 15 minutes dark were applied and the increase in the current intensity after 1 min illumination was taken as the analytical signal. The inhibition was calculated as: Inhibition (%) = $(I_0-I_1) / I_0*100$ (1), where I_0 and I_1- are the signals in the absence and in the presence of herbicide, respectively.

Results

The obtained AFM images of thylakoids from *Coccomyxa subellipsoidea* TL4 show disk shaped structures with heights of either 4-5 nm (corresponding to a horizontally sectioned thylakoid disk) or 10-12 nm (corresponding to a complete thylakoid disk) (see Fig. 1A). The AFM images of thylakoids from *Synechococcus elongatus* PCC 7942 showed similar disk-shaped structures (often stacked on top of each other). Higher magnification AFM images also revealed nicely organized, 20 nm diameter protein complexes, protruding 1-3 nm above the lipid layer of the thylakoid disk (see Fig. 1B).The images are in good agreement with the literature.

Inhibition investigation was conducted for whole cells, thylakoids and photosystem II (PSII) of both species. The presence of 60 ppb diuron caused a 14.9±8.6 %, 25.5±0.17 %, 25.8±9.3 inhibition of the photosynthetic activity of whole cells, thylakoids and PSII, respectively of *Synechococcus elongatus* PCC 7942. For *Coccomyxa subellipsoide*a TL4 the same concentration of diuron induced a 10.2±5.3 % inhibition of the whole cell's photosynthetic activity and 40.2±7.3 % for thylakoids.

From the various stabilizers (glycerol, PEG 8000, mannitol, sucrose and DMSO) used with PSII from *Synechococcus elongatus* PCC 7942, glycerol was the most efficient. After being stored for 12 days at -20˚C and with glycerol, PSII still showed 57.0 ±4.0 % inhibition by 2.57 µM diuron, while PSII stored without glycerol was completely insensitive to diuron. Thylakoids from *Coccomyxa subellipsoide*a

TL4 were stabilized with sucrose 2M. After 6 days at -20˚, their photosynthetic activity was 55.6 % of its initial value, and inhibition by 60 µM diuron decreased from 40.2±7.3 % to 14.9±2.5 %.
Calibrations curves for diuron were also obtained using optimum chlorophyll concentrations for *Synechococcus elongatus* PCC 7942 (7.5 µg/mL) and *Coccomyxa subellipsoide*a TL4 thylakoids (85 µg/mL). The calibration experiment emphasized detection limits of 13 ppb and 6 ppb diuron for *Synechococcus elongatus* PCC 7942 and *Coccomyxa subellipsoide*a TL4 thylakoids, respectively.

Conclusion

The studies on different cellular preparations of *Synechococcus elongatus* PCC 7942 and *Coccomyxa subellipsoide*a TL4 showed their sensitivity to diuron, thylakoids representing the best compromise between stability and sensitivity for both microrganisms. Stability of the preparations needs further improvement. An immobilization method for a future disposable platform assay is under study.

References

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Figures

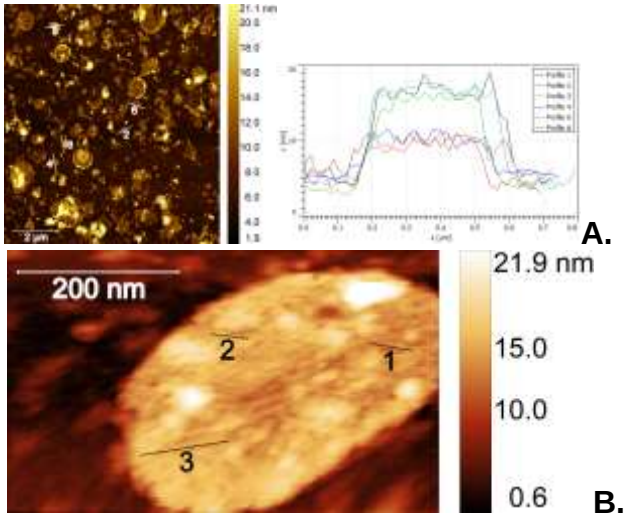

Figure 1. A. AFM image of several thylakoid disks from *Coccomyxa subellipsoidea* TL4 and cross sections highlighting the heights of such disks; B. AFM image of a single thylakoid disk from *Synechococcus elongatus* PCC 7942 showing semicrystalline arrays of complexes adjacent to disordered regions.

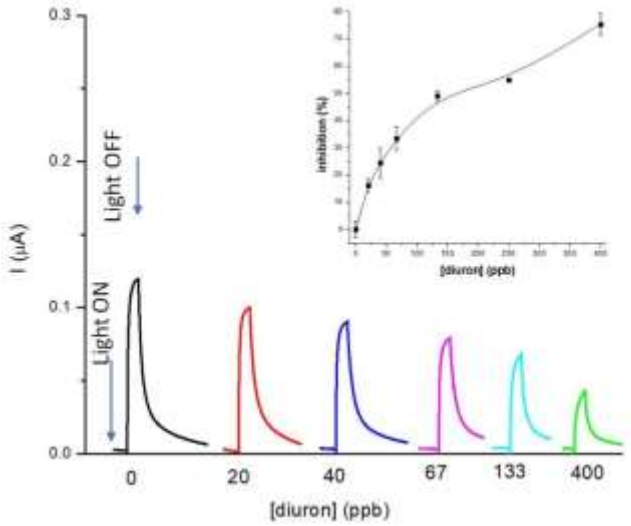

Figure 2. Photocurrents observed with *Synechococcus elongatus* PCC 7942 exposed to different concentrations of diuron. Inset: Calibration curve linking the extent of inhibition to the concentration of diuron.

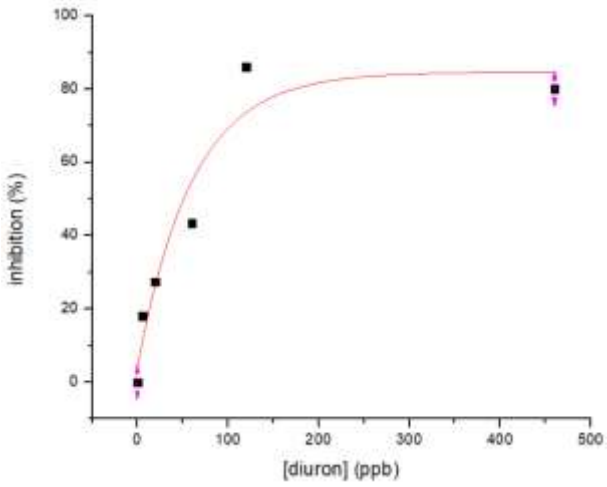

Figure 3. Calibration curve linking the extent of the inhibition of the photosynthetic current observed for *Coccomyxa subellipsoide*a TL4 thylakoids to the concentration of diuron

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