# Sensitivity of the ice cave microalgae *Coccomyxa subellipsoidea TL4* to marine water pollutants: potential for biosensors

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## Introduction

Pollution of marine bodies is a serious problem, aggravated in case of closed seas such as the Black Sea. One pathway to design cost-effective systems that can alert about the pollution due to agricultural runoff or intensive industrial activities close to the sea side relies on the inhibition of photosynthesis of microorganisms [1]. New phototrophs isolated from cold environments can provide the sensitivity and stability to low temperature required for on-site testing of marine water. The present study reports the characterization for the first time of the photosynthetic activity of microalgae *Coccomyxa subellipsoidea* TL4 isolated from the Scarisoara ice cave in Romania [2] as measured by an electrochemical sensor. The effect of water salinity and of various pollutants on the photosynthetic activity was examined with the aim of designing a biosensor for the screening of marine water. Artificial sea water (ASW) spiked with pollutants as well as real samples collected from the Black Sea were tested. Towards the development of a biosensor, the stability of thylakoids was investigated by freeze-frying in the presence of various stabilisers. Moreover, microalgae-impregnated paper was used in conjunction with screen-printed electrodes to devise a practical test for the on-site screening of marine water.

## Experimental

*Coccomyxa subellipsoide*a TL4 microalgae was isolated from Scarisoara ice cave, Romania and grown in liquid BG-11 at 20 ±3 °C for 3 weeks, until DO 660 nm exceded 0.8AU Cells were lysed by bead-beating and ultrasonication. The cell lysate was exposed to osmotic shock to disrupt residual intact chloroplasts, and the resulting mixture was loaded on a sucrose density gradient and subjected to ultracentrifugation [3]. Two fractions of thylakoid membranes were obtained (Figure 1), united and analysed by amperometry.

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Chronoamperometry experiments were conducted using a potentiostat from Princeton Applied Research equipped with EC-Lab software and a an ekectrochemical cell housing screen-printed electrodes (from Metrohm Dropsens, Spain). A white light lamp was used to illuminate the solution in the cell. The electrolyte was 20 mM phosphate buffer pH 7.1 containing 5g/L NaCl and contained thylakoids of *Coccomyxa subellipsoidea* solutions with chlorophyll concentration 85 µg/mL and the sample or calibration standard. was used to measure the current generated by the photosynthesis of the various biological preparations in presence and in absence of diuron. 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 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. Artificial sea water was prepared according to a literature protocol [4]

## Results

The *Coccomyxa subellipsoidea* C-169 cells had ellipsoidal shape with a diameter ranging from 4 to 10 µm (Figure 1). Thylakoids lyophilized in the absence and in the presence of stabilisers were tested after 1 week of storage at -20° C and compared to the fresh preparation prior to lyophilization. When no stabilisers were added the degree of inhibition by 60 ppb diuron dropped to an average of 10%, indicating significant degradation (Figure 2). Thylakoids stabilized with either 0.5M trehalose or 0.5M sucrose presented on average 58% and 63% inhibition in the same conditions, similar to values recorded with fresh preparations. Based on these results, papers impregnated with thylakoids and 0.5 M sucrose were stored in the fridge at 4° C and tested regularly.

The salinity of the medium (the electrolyte) greatly influenced the photosynthetic current, both by increased conductivity of the electrolyte and due to the effect on the thylakoids. It was found that the response was relatively stable in the range from 5 to 20 g/L NaCl. Further on, artificial sea water (ASW) was spiked with diuron and analysed. It was observed that the thylakoids' photosynthetic activity was not negatively affected by the ASW and that the sensitivity to diuron was very similar to that in the 20 mM phosphate buffer pH 7.1 with very low salinity. A 10 ppb diuron solution in ASW caused an inhibition of 26% of the photosynthetic activity (Figure 3). Sensors ontained from paper impregnated with thylakoids had lower sensitivity than thylakoids in solution, due to both the diffusional barrier brought by the paper and lower chlorophyll concentration in the cell (Figure 4).

## Conclusion

While optimization is underway, both use of free-dried thylakoids for tests in solution and thylakoid impregnated paper appear as valid solutions for fast testing of marine waters. Surprisingly, while CS was isolated from an ice cave, is not sensitive to salinity (at least up to 20 g/L, the maximum level in the Black Sea). Its sensitivity to diuron and heavy metals shows promise for the development of biosensors as early warning systems for screening the quality of marine water close to coastal area characterized by intense agricultural or industrial activities.

## References


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## Figures

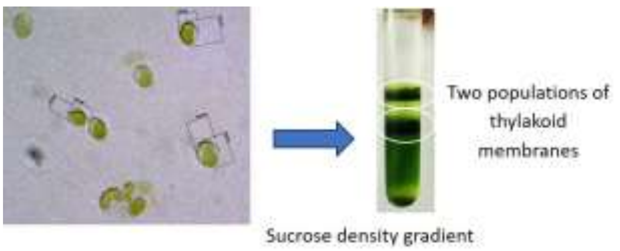


**Figure 1.** Phototrophic cells of *Coccomyxa subellipsoidea* C-169 observed by light microscopy and the fractions with thylakoid membranes separated by sucrose gradient .

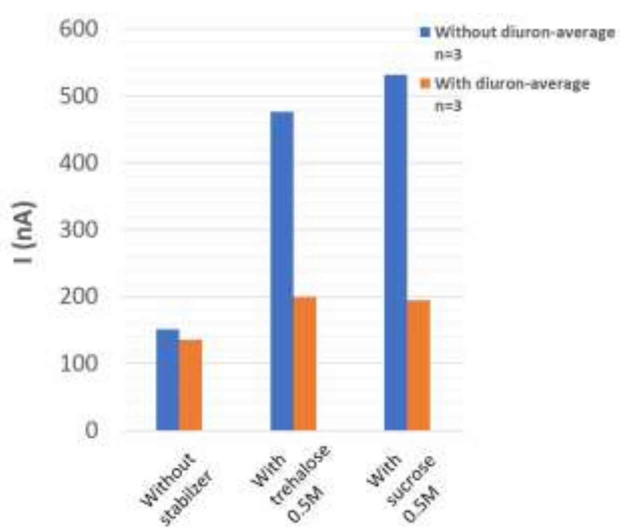


**Figure 2.** Sensitivity to 60 ppb diuron of lyophilized preparations of *Coccomyxa subellipsoidea* C-169 obtained in the absence and presence of stabilisers.

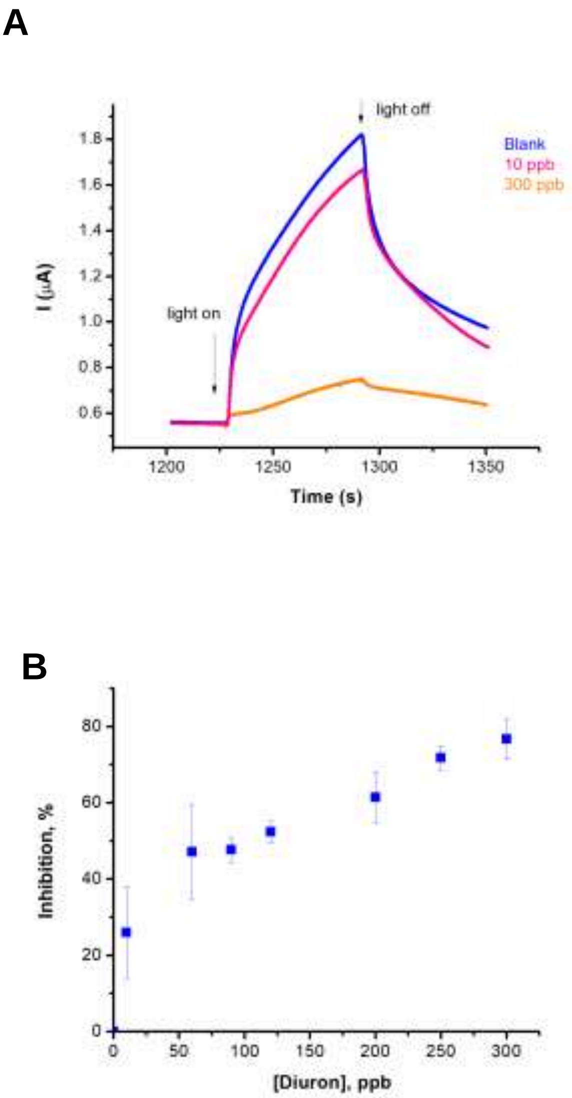


**Figure 3.** A. Typical amperometry signals obtained with thylakoids of CS tested in solution with a CNT electrode polarized at +0.4 V vs Ag in 0.7 mM hexacyanoferrate (II), in the presence of various concentrations of diuron in artificial sea water. B. The calibration curve for diuron in ASW.

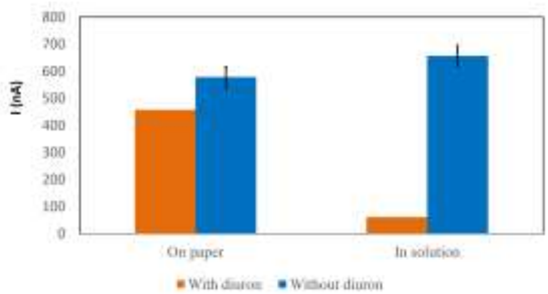


**Figure 4.** Photosynthetic activity of *Coccomyxa subellipsoidea* TL4 thylakoids in solution an on paper and their sensitivity to 60 ppb diuron

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