Supplementary Material (ESI) for Analytical Methods

## *Electronic supplementary information*

# A whole-cell amperometric herbicide biosensor based on magnetically functionalised microalgae and screen-printed electrodes

Alsu I. Zamaleeva,[a1] Ilziya R. Sharipova,[a1] Rezeda V. Shamagsumova,[b] Alexey N. Ivanov,[b] Gennady A. Evtugyn,[b] Dilara G. Ishmuchametova,[a] Rawil F. Fakhrullin[a*]

*[a] Biomaterials and Nanomaterials Group, Department of Biochemistry, Kazan (Idel buye/Volga region) Federal University, Kreml uramı 18, Kazan, 420008, Republic of Tatarstan, RF; Tel: +78432337833; E-mail: kazanbio@gmail.com*

*[b] Department of Analytical Chemistry, Kazan (Idel buye/Volga region) Federal University, Kreml uramı 18, Kazan, 420008, Republic of Tatarstan, RF*

*[1] Both authors contributed equally to this study*

To establish the optimal conditions for signal measurements, first cyclic voltammograms of ferricyanide ions were recorded. The mixture was prepared by mixing 90 μL of 0.1 M $Na_2SO_4$, 10 μL of 0.1 $K_3[Fe(CN)_6]$ and 100 μL of microalgae dispersion followed by spreading an appropriate aliquot onto the electrode surface. The voltammograms were recorded in daylight and in the dark (Fig.1S). The ferricyanide ion showed reversible behavior and quite a stable response. The peaks on voltammograms corresponded to reversible reduction and oxidation of $[Fe(CN)_6]^{3-/4-}$ pair. Both the peak currents and potentials remained stable in multiple cycling. Thus no diffusional limitation was observed in DC mode. For the two consecutive cycles recorded in light and dark conditions, the current regularly increased due to the competitive involvement of the ferrycianide ion in the electron transfer chain in the living algae cells. However, the changes did not exceed 5% of the peak current value and were too small for quantification of the effect.

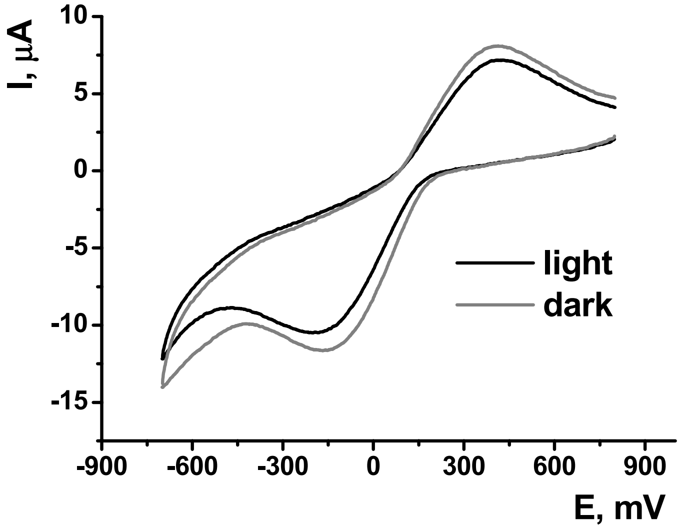


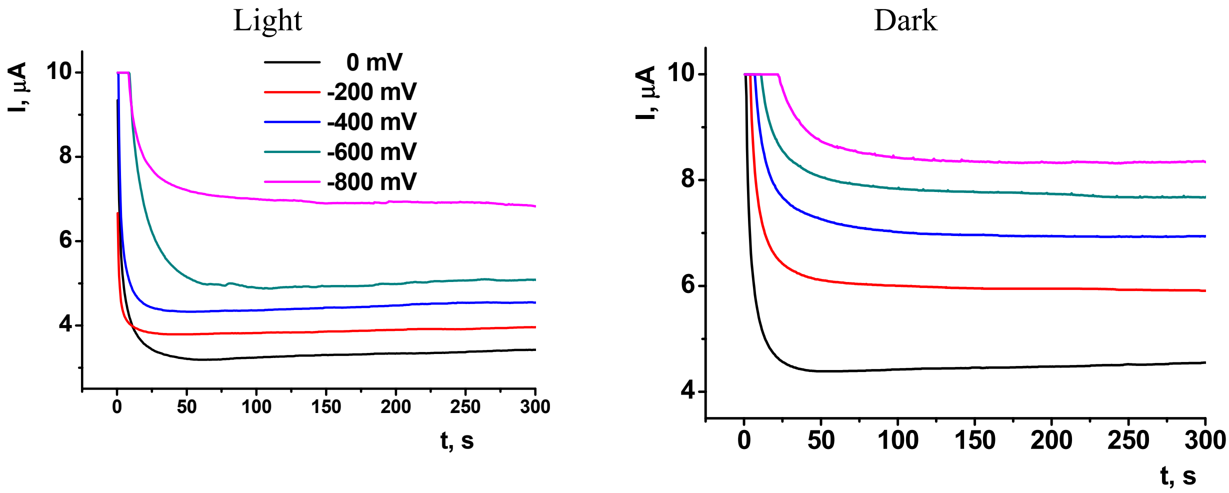
Figure 1S. Cyclic voltammograms recorded on screen-printed electrode after spreading 60 μL of mixture obtained by mixing 90 μL of 0.1 M $Na_2SO_4$, 10 μL of 0.1 $K_3[Fe(CN)_6]$ and 100 μL of microalgae dispersion

Similar effects were obtained for other ratios of microalgale and ferricyanide solution (data not shown). It should be mentioned that the reduction current increased in all the cases to some extent, higher than the anodic current. This coincides well with the function of ferricyanide ion as an artificial electron acceptor in photosynthesis reactions.

After that, the chronoamperomtric measurements were performed to find the working conditions for signal measurement. The electrode was polarized at the cathodic potential related to reduction of $[Fe(CN)_4]^{3-}$ and the cathodic current was recorded until stabilized (within 3-5 min). The appropriate curves are presented in Fig. 2S. Both in light and dark the steady-state values of cathodic currents increase with the working potential. However, the maximal difference in the signal was observed at -600 mV.

The results of the direct current voltammetry and chronoamperometry allowed us to conclude that the maximal difference in the signals related to dark and light were observed on the cathodic branch of the curves at -600 mV. Therefore we suggest the following mechanism of the signal generation. Ferricyanide ion $[Fe(CN)_6]^{3-}$ is cathodically reduced to ferrocyanide $[Fe(CN)_6]^{4-}$ which acts as an artificial electron acceptor in photosynthetic path. As the biochemical conversion is rather fast, this results in an increase of the appropriate current reflecting the concentration of the product of electron transfer near the electrode surface. Cyclic voltammograms are less sensitive to such additional way of the generation of oxidized species because of the direct oxidation of $[Fe(CN)_6]^{4-}$ on the electrode. Cathodic polarization of the working electrode at -450…-6700 mV is also used in other systems developed for fast estimation of microbial and photosynthetic activity (BOD measurements [L.Liu, L.Shang, C.Liu, C.Liu, B.Zhang, S.Dong, A new mediator method for BOD measurement under non-deaerated condition.// Talanta 81 (2010) 1170-1175], herbicide detection [I.Shitanda, K.Takada, Y.Sakai, T.Tatsuma, Compact amperometric algal biosensors for the evaluation of water toxicity.// Anal.Chim.Acta 530 (2005) 191-197, Shitanda, S.Takamatsu, K.Watanabe, M.Itagaki Amperometric screen-printed algal biosensor with flow injection analysis system for detection of environmental toxic compounds.// Electrochimica Acta 54 (2009) 4933-4936].

On the other hand, the whole chronoamperomgrams as shown in Fig. 2S can be used to detect the contribution of photosynthesis even though the stability of this response seems rather modest.



Dark – Light Difference Signal

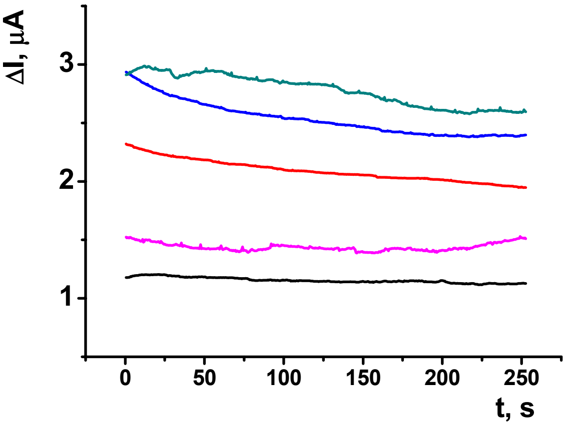


Figure 2S. Chronoamperograms recorded at different potentials on screen-printed electrode covered 60 μL of mixture obtained by mixing 90 μL of 0.1 M $Na_2SO_4$, 10 μL of 0.1 $K_3[Fe(CN)_6]$ and 100 μL of microalgae dispersion. Cathodic currents are plotted against the polarization period

To improve the reproducibility of the signal, it was suggested to use the periodical change of the illumination regime with recording the current with the same algae drop placed onto the electrode. Figure 3S shows the currents recorded at various potentials corresponded in accordance with Fig.2 conditions (the curves are shifted against each other to make clear the difference). The potential of -600 mV yielded the maximal difference in the currents corresponded to light and dark periods similarly to chronoamperomtry. Meanwhile, the use of rather short lighting period (30 s) makes it possible to average the response and hence improve its reproducibility.

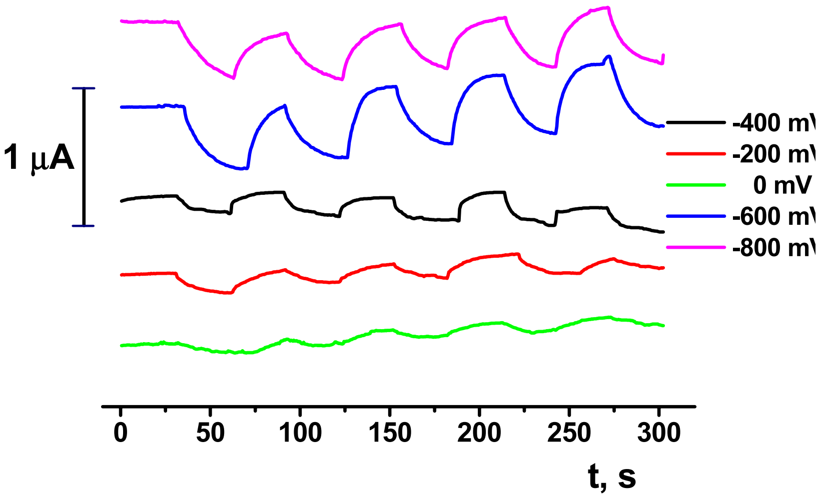


Figure 3S. Changes in the current through cyclic lighting the electrode by daylight (30 s dark and light periods) recorded at stationary potentials from 0 to -800 mV (measurement conditions as those for the data in Fig. 2S). The current curves are vertically shifted against each other to illustrate the difference in the signals.