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Research Note

Using the Green Flagellate *Euglena gracilis* Klebs as Physiological Dosimeter: is a Long-term Bioassay More Significant than a Short-term One?

Roman A. DANILOV*, Nils G. A. EKELUND
Department of Applied Science, Mid Sweden University , 871 88 Härnösand, SWEDEN

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Abstract : The use of short-term tests with *E. gracilis* Klebs in order to assess toxicity cannot be viewed as absolutely preferable. At least in the case with wastewater from the pulp and paper industry, 7 day-tests could lead to more explanatory and significant results.

Key Words: *Euglena gracilis*, bioassay, wastewater

Introduction

Algal toxicity tests have been in common use in Europe for a long time. Algae are a group of organisms especially suitable for bioassays due to their abundance in aquatic habitats and sensitivity to environmental pollution (Walsh and Merrill 1984, Tubak *et al.* 1996). Thereby single tests have gained the most popularity due to their simplicity and good value. Growth rate inhibition tests are those commonly used (Nyholm and Källqvist 1989). Additionally, other physiological parameter of algae have been reported to be promising in toxicity assessment. Häder *et al.* (1997) proposed to use parameters such as motility and cell shape in *Euglena gracilis* Klebs for reliable monitoring of water toxicity.

There seems to be a general agreement that short-duration (3-4 days) bioassays using unicellular algae are preferable to those of long duration (6-7 days), (Walsh and Merrill 1984, Nyholm 1985). But the question as to whether the short-duration tests are also favourable at lower concentrations of toxic substances, remains unanswered. However, during monitoring of the cleaning system efficiency, e.g., by the pulp and paper industry, the situation arises when low concentrations of toxic substances also need to be detected. During continuous load into the aquatic environment, they can accumulate and cause long-acting damage to aquatic biota (EPA 1997). Therefore it is important to test and eventually calibrate existing bioassay methods concerning their significance at lower concentrations of toxic substances.

In accordance with the statements above, we performed experiments with *E. gracilis* in order to compare the significance of the short-term and long-term approach when assaying the toxicity of wastewater from the pulp and paper industry before and after cleaning (Table). Strain Z of *E. gracilis* was used (Department of Plant Physiology, University of Lund, Sweden). The cells were grown in a freshwater medium (Checcucci *et al.* 1976) in 100 ml Erlenmeyer glass flasks at 20°C in a cultivation cabinet (Termax Klimatskåp, 6395 F/FL, Ninolab AB). Starting density was held at ca 10^4 cells/ml. The light/dark cycle was 16 h/8 h with an irradiance of 19 W m^{-2} (400-700 nm). Wastewater samples were taken from the cleaning system (before and after the last step in the cleaning process) of the paper factory SCA (Svenska Cellulosa Aktiebolaget) in the Paper Mill, Ortviken, Sweden. The samples were filtered in order to remove small wood particles (filterpaper 595, Schleicher & Schuell, Germany). The cells were grown at different concentrations (1:20, 1:10, 1:5, 1:2, 1:1 and undiluted) of cleaned and uncleaned samples of wastewater in batch

* Author for correspondence: e-mail: roman.danilov@tnv.mh.se, fax: +46 611 86160, phone: +46 611 86292

Table. Influence of cleaning process on content of common ions and elements in the samples

Matters	Before cleaning, mg/l	After cleaning, mg/l
pH	6.70	7.60
NO_3^-	0.100	0.203
P_{total}	0.200	0.258
K^+	19.8	17.9
Mg^{2+}	5.77	4.64
S_{total}	148	140
Ca^{2+}	160	73.5
Cl^-	20.7	14.4
Mn_{total}	2.92	1.94
B_{total}	0.073	0.045
Cu^{2+}	0.016	0.061
Fe_{total}	1.77	1.60
Zn^{2+}	0.063	0.279
Mb_{total}	0.003	0.03
Al_{total}	0.124	0.096
Si_{total}	4.58	7.19
NH_4^+	1.200	0.702

cultures. For each concentration studied, the measurements were repeated three times and mean values from three replicates were calculated. The measurements were performed on the fourth and seventh days. In order to measure their motility and cell shapes, the cells of *E. gracilis* were studied with the aid of a compound microscope, Nikon Optiphot, connected to a video CCD Camera Ikegami ICD-44L (Ikegami Tsushinki Co., Ltd., Japan). The image was translated to a PC. All calculations were done with Motile System V. 1.7 (motility) and Count System V. 2.4 (cell shape) software. Both were developed by D.-P. Häder and K. Vogel, Erlangen, Germany. The motility was measured as μm s^{-1}. The shape of the cell was expressed as a form factor which showed the deviation of the cell shape from the form of an ideal ball (form factor equal 1). When measuring the motility, the average value from 1000 measurements was calculated in each case. Calculations of average values for cell shape were based on 40 cells observed in each case. Statistical analyses using Tukey's method (*t*-test) for calculating confidence intervals for all pairwise differences between level means were performed with the package MiniTab 11. The family error rate used was 0.05.

We found that the duration of experiments (4 or 7 days) did not influence the coefficient of variation (CV) at the same concentrations of waste substances both in cell shape and motility and in the range from 18 to 32%. In general the significance of the cell shape as physiological factor was very low according to the *t*-test and did not depend on the duration of experiments. Thus the cell shape of *E. gracilis* seems to be an unreliable parameter at lower concentrations of wastewater substances from the pulp and paper industry in contrast to the results reported by Häder *et al.* (1997). On the other hand, the significance of changes in motility due to changes in the concentrations of wastewater both before and after cleaning was high and increased by 20% at the seventh day compared to the fourth day. Therefore, it was not possible to obtain on the fourth day any clear trends in motility in relation to the changing concentrations of the samples tested. However, on the seventh day clear trends were obtained, supported by the *t*-test (Danilov and Ekelund, unpublished results).

We conclude that the use of short-term tests with *E. gracilis* in order to assess toxicity cannot be viewed as absolutely preferable. At least in the case with wastewater from the pulp and paper industry 7 day-tests could lead to more explanatory and significant results.

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