Available online at www.sciencedirect.com
ScienceDirect
Ecotoxicology and Environmental Safety 69 (2008) 254–262
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Short-term effects of benzalkonium chloride and atrazine on *Elodea canadensis* using a miniaturised microbioreactor system for an online monitoring of physiologic parameters

Marco Vervliet-Scheebaum*, Raphael Ritzenthaler, Johannes Normann, Edgar Wagner

Institute of Biology, University of Freiburg, Schaenzlestrasse 1, D-79104 Freiburg, Germany

Received 3 July 2006; received in revised form 30 January 2007; accepted 2 February 2007
Available online 11 April 2007

Abstract

The study evaluated the effects of benzalkonium chloride (BAC) and atrazine on the macrophyte *Elodea canadensis* (Michaux) using a miniaturised monitoring test system consisting of a microbioreactor of reduced volume and integrated sensors for the online measurement of physiologic parameters, like oxygen production and different parameters of fluorescence. Different concentrations of both chemicals were applied to leaves of *E. canadensis* and the physiologic endpoints evaluated after 1 h. A concentration-dependent reduction of the oxygen production and of the effective quantum yield of energy conversion was recorded. The mini-PAM technique implemented in the presented system allowed for a clear monitoring of the kinetic of BAC and atrazine, showing their distinct mode of action. No observable adverse effects were recorded up to concentrations of 2.5 mg/L and 10 μg/L, for BAC and atrazine, respectively. These values are in accordance with available results in the literature, hence indicating that the microbioreactor test system might be suitable, on the one hand, for the laboratory screening of potential short-term toxicity of contaminants on aquatic plants, and on the other hand, serve as an in situ field biomonitoring system for the rapid detection of pollutants in water.

Keywords: Benzalkonium chloride; Atrazine; Macrophytes; Microbioreactor; Monitoring; *Elodea canadensis*; Fluorescence measurements; Physiologic parameters

1. Introduction

Irresponsible and uncontrolled use of contaminants, as well as accidental spills can pose serious risks to the environment. A correct evaluation of their toxic potential is, therefore, one of the main goals in ecotoxicology. Suitable experimental set-ups and test designs are necessary to allow for a proper assessment of the potential toxicity of contaminants (Caquet et al., 2000). Unfortunately, for an assessment of potential risks on macrophytes in the aquatic environment few guidelines and experimental designs have been implemented or recommended so far. The protection of the so-called 'non-target aquatic plants', i.e. those plants outside the treatment area where injury would be commercially or aesthetically undesirable (McKelvey et al., 2002); however, is crucial, since they serve as food and shelter for animals, both terrestrial and aquatic species. In aquatic ecosystems, the role of aquatic plant species is even more important, since they are the primary producers at the lowest trophic level, producing oxygen, controlling the nutrient cycling and the water quality, as well as stabilising the sediment and serving for shelter to the various organisms of the aquatic medium (Grace and Wetzel, 1978; Lewis, 1995). It is also known that plants have considerable influence on fate and behaviour of pollutants in surface waters (Roy and Hänninen, 1994; Pflugmacher and Steinberg, 1997; Hand et al., 2001).

To assess the potential risks of chemicals entering aquatic ecosystems and potentially endangering aquatic non-target plants a series of laboratory single species tests, basically on algae, are required (Brock et al., 2000). For the evaluation of herbicide toxicity only the duckweed *Lemna*

*Corresponding author. Fax: +49 761 203 2840.
E-mail address: marco.vervliet@biologie.uni-freiburg.de (M. Vervliet-Scheebaum).

0147-6513/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2007.02.001

sp. is tested as a standard macrophyte species. Alternative macrophytes have been proposed for laboratory studies (Swanson et al., 1991; Wang, 1992; Lovett Doust et al., 1994; Lewis, 1995; Wang and Freemark, 1995; US EPA, 2001; Knauer et al., 2006), or for field studies in either micro- or mesocosm tests (Van den Brink et al., 1995, 1997, Van Geest et al., 1999), but implementation into regular testing schemes is still far away.

The parameters for the evaluation of aquatic plants have been based mostly on growth, like biomass or length increase (Vervliet-Scheebaum et al., 2006). The assessment of such endpoints usually takes a couple of days, weeks or even months, their use therefore is very limited if a rapid evaluation of potential toxicity, e.g. after accidental spills, is needed or, if an 'in situ' characterisation of toxicity is required. Being time consuming, the costs for such investigations tend to increase heavily with test duration.

Fast biological responses evoked by contaminants can be observed by continuous monitoring of the physiological status of an organism. So far, these aspects have not found significant implementation in the aquatic risk assessment of chemicals, e.g. pesticides. Using the physiological parameters as endpoints for an evaluation of potential negative impacts might allow for a better insight into the kinetics and mode of action of compounds once they impact on the organism.

The miniaturised microbioreactor system presented here was developed in order to fill this gap (Wolf et al., 1998; Eich, 2001). Several parameters related to the physiology of organisms, e.g. oxygen production or consumption, changes in the medium, like pH or conductivity, fluorescence and others, can be recorded in parallel and 'in vivo' in this test systems, without damaging the test objects. The monitoring with non-invasive, physical sensors can additionally be combined with different optical methods, e.g. confocal laser scanning microscopy and fluorescence probes for the monitoring of metabolic activities in vivo. The dimension of the microbioreactor (with a volume of in-between 150 and 650 μL) might seem very limiting at first sight in relation to the size of the testing material. It allows, however, to extent its area of use to many fields, such as medical applications or cellular physiology.

For the experiments reported here, we selected the macrophyte *Elodea canadensis* (Michaux), a wide-spread species present in many aquatic systems and previously used in different set-ups to evaluate potential toxicity of chemicals on non-target aquatic plants (Dabydeen and Leavitt, 1981; Rodgers, 1991). An assessment of the potential impact of contaminants on the physiologic status of *E. canadensis* was done monitoring the changes in the oxygen production, in the pH of the medium and in fluorescence. Chlorophyll fluorescence measurements were performed using the pulse-amplitude modulated (PAM) fluorimeter technique, which has been proven to be suitable for chlorophyll fluorescence yield recordings at largely varying ambient light intensities (Schreiber, 1986, 1996). To allow for a comparison of the results obtained in the microbioreactor with studies performed earlier, we selected the biocidal compound benzalkonium chloride (BAC) and the triazine herbicide atrazine, two chemicals that are used or have been used worldwide and possess distinct modes of action. They were thus supposed to display specific effects on the physiology of *E. canadensis*.

2. Material and methods

2.1. Technical details and design of the microbioreactor system

The microbioreactor system (Fig. 1) is conceived as a flow-through measuring device, consisting of a small bioreactor with a volume between 150 and 650 μL as central unit, and integrated sensors for the parallel measurement of parameters, like pH, O_2 and temperature. Optical windows at top and bottom of the bioreactor allow for illumination of the test objects with a cold light source from above, and for fluorescence measurements using a pulse amplitude modulated (mini-PAM) fluorimeter from below (see Table 1 for details) (Walz, 1999).

Medium is constantly supplied to the bioreactor via a pump and a tube system. A bypass provides medium directly to the sensors without passing the bioreactor; thus measuring the parameters (O_2 and pH) directly in the medium as control/reference values. The medium flux is controlled by a valve via a computer, which monitors the whole system online and records the data. Table 1 gives a summary of all the materials and of the equipment used in the laboratory testing.

Prior to the start of a test, a calibration of the sensors is required. For both sensors, pH and O_2, this is done following a two-point calibration. Two buffer solutions with a pH differing by more than three full pH-units are pumped consecutively through the bypass to calibrate the pH-sensor. The output value indicates the sensitivity of the pH-sensor as a percentage (0–100%) of a set default value measured in (mV/pH). For the calibration of the oxygen sensor the sensor is unplugged from the block for the first calibration point (equivalent to 0 mg/L O_2). Once the sensor is plugged in, the dissolved O_2-concentration of the test medium is used as the second reference value. The O_2-concentration dissolved in aqueous solutions at a defined temperature can be obtained from appropriate tables (e.g. Oehme and Schuler, 1983). The sensitivity of the O_2-sensor is indicated in (pA × L/mg). Values over 20 pA × L/mg indicate a proper measuring sensitivity of the sensors for testing. Further details for the storage, cleaning and regeneration of the sensors can be found in Eich (2001). For the measurement of the fluorescence, the fiber optic of the mini-PAM is placed at approximately 8 mm from the lower optical window of the microbioreactor. For a zero point estimation the fibre optics of the mini-PAM system is calibrated using an empty microbioreactor and pressing the AUTO-ZERO of the device. The settings of the mini-PAM were adjusted as follows, measuring frequency: 0.6 kHz (low frequency); measuring light: off; saturating-width: 0.8 s; saturating intensity: 8; measuring intensity: 3. Once the measurements are started the fluorescence of *E. canadensis* is registered by pressing the start button. Following parameters were recorded and further evaluated: F_t (fluorescence yield, measured briefly before triggering of the saturating light pulse), $F_{m'}$ (maximal fluorescence, reached during the saturating light pulse), $(F_{m'}-F_t)/F_{m'}$ (effective quantum yield calculation of photochemical energy conversion, i.e. efficiency of photosynthesis) (see Walz, 1999 for details).

2.2. Test organism and culturing conditions

E. canadensis (Hydrocharitaceae) was obtained from a local supplier of aquarium plants. The macrophytes were cultured in 20 L aquaria under constant light conditions (40 μmol/m^2/s) at 25 °C in a medium containing $CaCl_2 \times 2H_2O$ (91.7 mg/L), $MgSO_4 \times 7\ H_2O$ (69 mg/L), $NaHCO_3$ (58.4 mg/L) and $KHCO_3$ (15.4 mg/L), according to Smart and Barko (1985). Sand was introduced in the aquarium to anchor the *E. canadensis* shoots.