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ScienceDirect
Chemosphere 66 (2007) 1329–1335
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

Quantification of ecotoxicological tests based on bioluminescence using Polaroid film

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Received 21 September 2005; received in revised form 27 June 2006; accepted 10 July 2006
Available online 1 September 2006

Abstract

Assays based on the measurement of bacterial luminescence are widely used in ecotoxicology. Bacterial strains responding either to general toxicity or specific pollutants are rapid, cost-effective and easy to use. However, quantification of the signal requires relatively expensive instrumentation. We show here that the detection of luminescence of BioTox™, a *Vibrio fischeri*-based toxicity test, and of a specific recombinant bacterial strain for arsenic determination, is possible using common Polaroid film. The exposed films can be used for visual or computer-assisted quantification of the signal. Qualitative visual comparison to standards can be used in the rapid and relatively accurate estimation of toxicity or pollutant concentration. The computer-assisted method significantly improves the accuracy and quantification of the results. The results obtained by computer-assisted quantification were in good agreement with the values obtained with a luminometer.

Keywords: Bioreporter; Environmental analysis; Toxicity testing; Arsenic; Ground water; Drinking water

1. Introduction

Assays based on the measurement of bacterial luminescence are widely used in ecotoxicology. One of the most commonly used applications is the measurement of inhibition of luminescence of a luminescent marine bacterium *Vibrio fischeri* (Ribo and Kaiser, 1987). Luminescence is connected to the metabolism of bacteria and only those bacteria having an active energy metabolism produce luminescence. Accordingly, when metabolism is obstructed or totally stopped (death of the bacteria) by a toxic sample the level of luminescence decreases. This phenomenon is utilised in exotoxicological tests based on the inhibition of bioluminescence of bacteria. The test based on *V. fischeri* strain NRRL B-11177 is available under different trade names such as MicroTox™ (Azur Environmental, United States), ToxAlert™ (Merck, Darmstadt, Germany) and BioTox™ (Aboatox, Turku, Finland) and it has been adopted as an official standard in several countries (ASTM method D5660-1995; DIN38412-1990; ISO 11348-3-1998). Tests based on *V. fischeri* are non-specific as any toxic effect caused by the sample is detected while no information on the identity of the pollutant is obtained.

More recently, specific and semi-specific luminescent strains have been developed for the detection of a number of environmental pollutants such as organic compounds (King et al., 1990; Applegate et al., 1998), organometallic compounds (Ivask et al., 2001), inorganic ions (Virta et al., 1995; Tauriainen et al., 1997), oxidative stress (Belkin et al., 1996), and genotoxicity (van der Lelie et al., 1997). They are based on the controlled expression of reporter genes such as the *lux* operon, which encodes the genes for the bacterial luminescence-producing system. Such an expression is controlled by a transcriptional regulatory protein which specifically recognizes the target analyte. When the target is present, it interacts with the regulatory protein and the complex activates the expression of the *lux*

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doi:10.1016/j.chemosphere.2006.07.034

operon, hence the level of luminescence increases. In contrast to the *V. fischeri* test, the presence of a target compound results in increased bacterial luminescence even before the analyte concentration reaches a toxic level. These bacteria provide information about the identity of the contaminant and in some cases also an estimation of the concentration of the target compound. An important feature is that they measure bioavailability of their target analyte, which is difficult to measure with traditional chemical methods but is extremely important in the assessment of environmental risk caused by a toxic compound (Daunert et al., 2000; Ehlers and Luthy, 2003; Semple et al., 2004).

The advantages common to all measurements performed using luminescent bacteria are low price and simplicity of measurement. Luminescent bacteria are well suited for measurements performed under simple, even field conditions. There are however both financial and organizational limitations in the use of luminescent bacteria that reside mainly in the quantification of their light emission. Although there is a wide selection of luminometers available commercially, the capital cost of even the most inexpensive model is still relatively high, especially for authorities and scientists in developing countries.

X-ray film has been used to detect luminescence from colonies of bacteria expressing the *lux* operon (Wood and DeLuca, 1987) and a photograph of bioluminescent bacteria on 96-well plates has been presented without experimental details (Simpson et al., 2001). Obviously the detection of luminescence should be possible using normal photographic film. In this study we investigate the possibility of quantifying luminescence from luminescent bacteria using a normal Polaroid film which is widely available. The film was exposed in a simple dark chamber and immediately used for visual approximation of the signal. Furthermore, the film was subjected to computer-aided quantification of the signal for more accurate results and the results were compared with values acquired by luminometric measurements. The results were obtained using a BioTox™ kit (a MicroTox™ equivalent) and a specific luminescent sensor for arsenic.

2. Experimental section

2.1. Materials

$ZnCl_2$(⩾98% pur.) was purchased from Fluka and $NaAsO_2$ from Sigma (⩾98% pur.). Water was purified with MilliQ equipment (Millipore, Bedford, MA). Aboatox BO1243-500 BioTox™ Kit, containing lyophilised *V. fischeri* NRRL B-11177 was purchased from Aboatox Ltd (Turku, Finland). The specific strain for arsenic *Escherichia coli* MC1061(parsRlux) has been described previously (Hakkila et al., 2002).

A prototype exposure chamber supplied by Polaroid Corp. (Waltham, MA) was used for the exposure of the films. The apparatus can hold one standard eight-well strip of an 96-well plate. Eight-well strips were EIA/RIA from Costar. The film used was Polaroid 600 gloss/brilliant instant.

The work with arsenic was carried out in a ventilation hood, using the required safety equipment. The waste containing arsenic was treated according to institutional guidelines.

The environmental samples (KJ1, KJ2, Ki5, KJ6, KJ7, KJ8 and KJ10) for the BioTox™ measurements were ash heap leachate from the oil shale regions of Kohtlajärve and Kiviõli, Estonia. Samples KJ2, Ki5, KJ7 and KJ8 were diluted 1/25-fold and sample KJ6 1/5-fold in 2% w/v NaCl solution to decrease their toxicity to *V. fischeri*. The environmental samples for the arsenic determination were ground water samples from Utah, USA. The samples were collected into plastic containers without any further treatment and stored at 4 °C.

2.2. Cultivation and freeze-drying of sensor bacteria for arsenic measurement

Bacteria for arsenic measurement were cultivated in M9 minimal medium supplemented with 0.5% casaminoacids and 100 μg ml^{-1} ampicillin on a shaker (300 rpm) at 37 °C. They were grown until the OD_{600} reached 0.7, after which they were harvested by centrifugation and resuspended into an equal volume of the same medium supplemented with 10% lactose. They were then lyophilised in 1.0 ml aliquots as descibed previously (Ivask et al., 2001) using a Lyofast S 04 freeze-dryer (Edwards Kniese & Co Hochvacuum GmbH, Marburg, Germany).

2.3. Preparation of BioTox™ reagents and bacteria for arsenic measurement

Lyophilised *V. fischeri* NRRL B-11177, provided in the BioTox™ toxicity kit, was rehydrated with 2% w/v NaCl solution for 30 min at 4 °C and then for 30 min at room temperature. Solutions of $ZnCl_2$ were mixed with 20% w/v NaCl provided with the kit, thereafter adjusting the NaCl concentration of $ZnCl_2$ standards to 2% w/v. The blank was a 2% w/v NaCl solution in distilled water.

The lyophilised *E. coli* strain MC1061(parslux) was rehydrated overnight with LB medium (Sambrook et al., 1989) at room temperature. The standard solution of $NaAsO_2$ was prepared in distilled water.

2.4. Measurement protocol for luminometric measurements

100 μl of the *V. fischeri* reagent were mixed with 100 μl of each $ZnCl_2$ dilutions in wells of a microtiter plate. After a brief shaking, the plate was incubated for 30 min at room temperature. During this time, to ensure the stability of luminescence, the plate was measured over five minute intervals until there were no longer significant changes in the luminescence values.