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Development of an automated biosensor for the detection of acute toxic effects of environmental pollutants

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Key words

biosensor; bacterial luminescence; continuous environmental monitoring; immobilized bacteria; *Vibrio fischeri*

Abstract

The change of bacterial bioluminescence is an efficient choice of application to determine the biological effects of single environmental toxicants and pollutant mixtures. We developed the biosensor *onlineTOX*® for application of immobilized bacteria. The biosensor was incorporated into a flow injection system. The aim was to establish a continuous working screening system for environmental pollutants in different water bodies such as surface water, sewage treatment plant intakes or outlets.

Introduction

For approximately 50 years, intact light-bacteria have been put in for toxicological examinations and for toxicological screening tests, too (1, 2, 3). Biosensors based on living organisms fulfil the qualifications of an effective environmental monitoring. Therefore luminescent marine bacteria in suspension were already used several times in the development of more sensitive tests for water monitoring (2, 3, 4, 5, 6, 7).

Since March 1991 in Germany the Luminescent bacteria test (DIN 38412 L34 [8]) is established as an official DIN method and since April 1999 as a DIN EN ISO (11348 [9]) for testing water, waste water and chemicals.

Our institute works on the development of environment-early-warning-systems. A toxicity analyser for fluid media that based on immobilized luminescent bacteria (*Vibrio fischeri*), which are incorporated into a flow injection system, was established. This biosensor could be developed up to the market-maturity.

The advantages of the luminescent bacteria are their high sensitivity on damaging environmental influences, the simple handling and the classification to the risk-group one and therefore a more safely dealing at the practice use.

Appliance construction and function manner

The toxicity analyser *onlineTOX*® (fig. 1 shows the front view of the apparatus in a climatic cabinet, fig. 2 shows the mode of function) exist of a biosensor, which is integrated in a flow injection system and allows sampling every 30 to 60 minutes. The difference of light emission after application of a control solution and after application of the sample is used as measurement of inhibition. In case of a system failure or transgression of a determined threshold value an alarm will be triggered and in the second case retaining samples will be taken. These samples enable a differentiated chemical analysis and can be taken in consideration for securing the originator of pollution.

The toxicity analyzer contains the following components: microbial biosensor based on stationary immobilized luminescent bacteria; a flow injection system containing a complex set up of magnetic valves and tubes; a microprocessor; a data logger; a display and a climatic cabinet.

Fig. 1 Front view of the toxicity analyzer onlineTOX® *in a climatic cabinet*

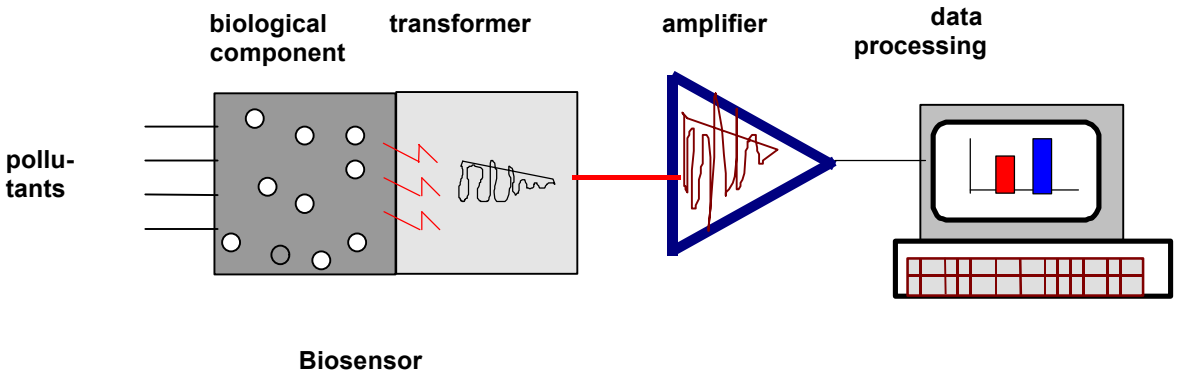

Fig. 2 Mode of function of the toxicity analyzer

The biological component of the toxicity analyzer is a chip with immobilized luminescent bacteria (10) that is in a tempered one-way cuvette. The change of light emission in the consequence of toxic impacts can be used as a measurement. An optoelectronic transducer (photomultiplier) transforms the emitted light into an electric signal and via a highly sensitive low noise DC-amplifier the data are transported towards an internal microprocessor and/or an external computer.
The supply of the different media takes place discontinuous (fig. 3). The luminescent bacteria were immobilized on a substrate in order to enable multiple measurements and to avoid a loss of bacteria. The samples to be measured as well as all required solutions for nutrient supply, oxygen supply and electrolytes are pumped into (⇨) the chamber with the immobilized bacteria and out of it (➡) via an set up of magnetic valves (MV) and tube pumps.

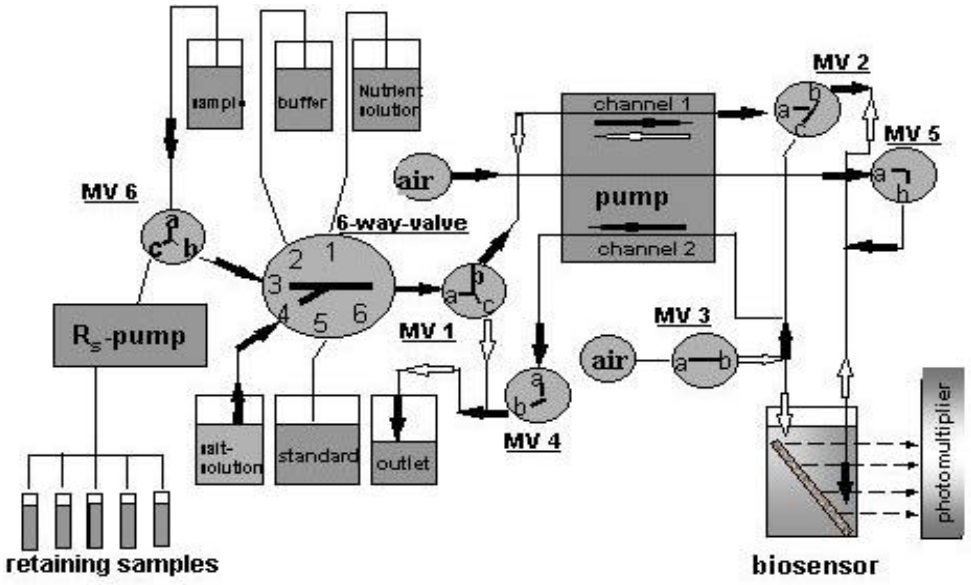

Fig. 3 Schematic description of the toxicity analyzer onlineTOX®

The control and dispersal of solution intakes is manages by a 6-way-valve and various magnetic valves (see fig. 3). Because of the discontinuous supply of the solutions two different operation phases can be distinguished: filling of the cuvette (pump works clockwise) and drainage (pump works counter clockwise). Both phases are explained in fig. 3. The control of the pumps and valves as well as calculation of the resulting inhibition is ensured by a microprocessor. An internal data logger saves the measured data. The installation of an external recorder is enabled. The devices memory program makes available different measurement and service programs.
The analyser works discontinuously. The advantages are a reduction of fluid consumption for nutrition, rinsing etc., a protection of mechanical parts of the apparatus (pump etc.), and prolongation of lifetime of the immobilized bacteria.
Calibration of the analyser is performed by a non-poisonous standard (concentrated salt solution). The measurement of an environmental sample takes place in accordance with the DIN EN ISO 11348 (9). A part of the sequence of a measurement curve is shown in the following graph (fig. 4). One

measurement cycle includes the measurement of a control and/or a standard to check-up the physiological conditions of the light-bacteria and the measurement of 6 or 12 environmental samples. At the end of one cycle a repetition of the measuring-cycle takes place up to the exchange of the bacteria because they are death or their light emission is to low. Before measuring the environmental sample it is enriched with a salt solution up to a concentration of 2 % NaCl. For an optimal supply of the bacteria with nutrient and to the extension of the lifetime of the immobilized cells after each measuring-cycle (control or standard and samples) a nutritional phase with all necessary nutrients takes place (fig. 4).
The measurement of bioluminescence occurs only through air so correction values for turbidity and/or colour of samples can be neglected.
The user has the possibility to establish certain measuring parameters like the incubation time of the examined sample and the static threshold value.

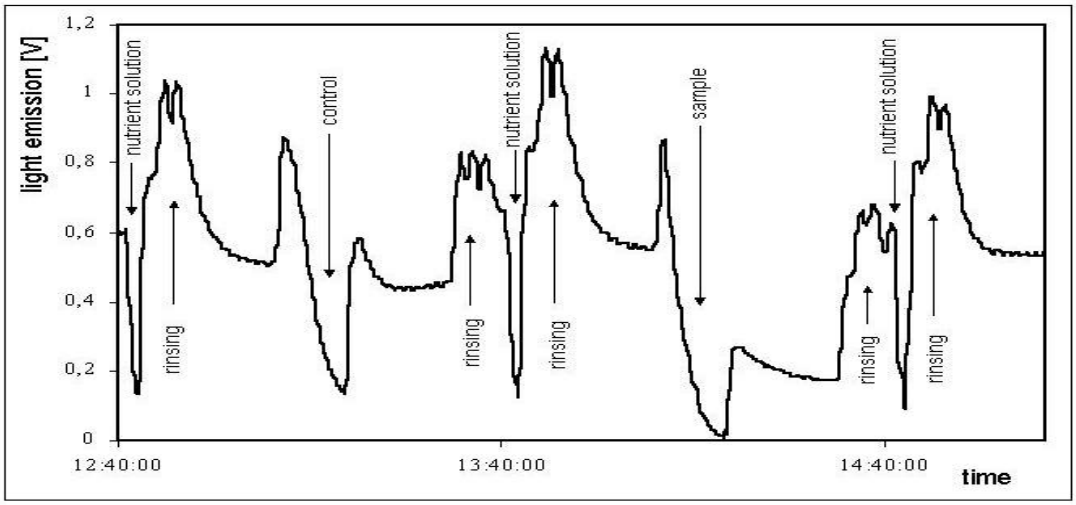

Fig. 4 Light emission of immobilized bacteria during measurement of one control and one sample

The controls of the pumps and valves as well as calculation of the resulting inhibition are ensured by a micro-processor. For input and output of dates and also for control of function there are analogous input ports (12 bit) and analogous output ports (8 bits) and digital output ports, too. The intern logger saves all measuring dates and can be read by the user via a matching unit (RS 232). Furthermore it is possible to read the analogous measurement dates directly and also the calculated inhibition values as analogous figures in the field from 0 to 10 V DC (agree 0 to 100 % inhibition). This allows the connection of a printer.

Results of testing of reference substances

To check up the sensitivity of *onlineTOX*® reference substances, for example heavy metals and organic pollutants, were tested parallel to the DIN EN ISO 11348.
In fig. 5 the inhibition curves of some tested substances are represented with an incubation time of 15 minutes on the immobilized cells in the *onlineTOX*®.

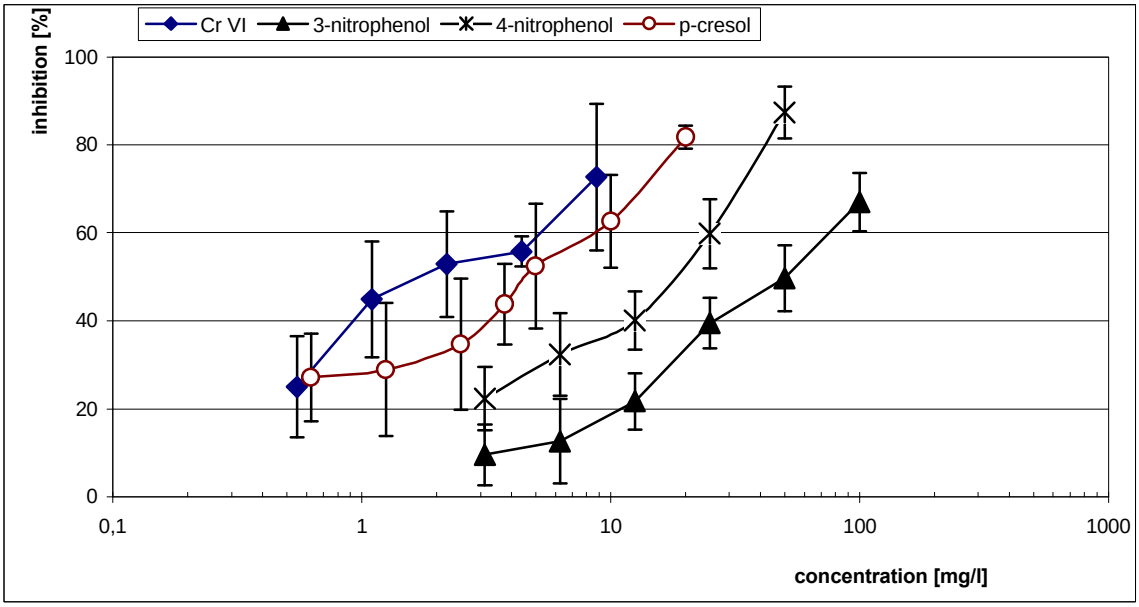

Fig. 5 Inhibition graphs of some tested chemicals with the onlineTOX®

Some more results are demonstrated in table 1. It is clear that in most cases the results (EC_{20}- and EC_{50}-values) of the toxicity analyzer are in good agreement with the results of the DIN-tests.

Table 1 Comparison of the EC_{20} and EC_{50} values obtained with the DIN EN ISO 11348-1 test and with onlineTOX® *using a reaction time for the reference substances of 30 minutes in the DIN test and of 15 minutes for measurements with the toxicity analyzer (n = number of measurements).*

Substance	DIN Test (30 min)		*onlineTOX*â (15 min)	n
Cr VI (as $K_2Cr_2O_7$)	EC_{20} =	0,34	0,27	10
	EC_{50} =	1,98	2,19	10
3,5-dichlorophenol	EC_{20}=	3,76	4,51	12
	EC_{50}=	6,52	8,67	12
p-cresol	EC_{20}=	0,51	0,60	10
	EC_{50}=	1,88	4,09	10
pentachlorophenol	EC_{20}=	0,80	1,30	36
	EC_{50}=	1,65	3,59	36
3-nitrophenol	EC_{20}=	13,1	9,41	31
	EC_{50}=	35,1	47,08	31
4-nitrophenol	EC_{20}=	3,93	3,53	28
	EC_{50}=	17,59	12,80	28
o-toluidine	EC_{20}=	0,08	2,33	36
	EC_{50}=	0,44	15,39	36

concentration [mg/l]

Presently, the *onlineTOX*® is tested at different practice locations, so for example in the outlets or intakes from sewage treatment plants, in flow-waters, with the supervision of leachate and in drinking water-processing-plants.

Fig. 6 shows excerpt-wise measuring results from the supervision of leachate over a time period of approximately 44 hours. The samples were filtered merely roughly before their testing.

In the total-time period, inhibitions from 0,5 to 43 % were measured as well as easy activations of the bioluminescence in three cases during the experiment. The retaining samples proved with the laboratory examination to be wise toxic (G_L-values in the DIN-test were 3 and 4 and small amounts of heavy metals and organic compounds such as PAH´s and biphenyls were measured, this in their sum the measured inhibition with big likelihood has caused).

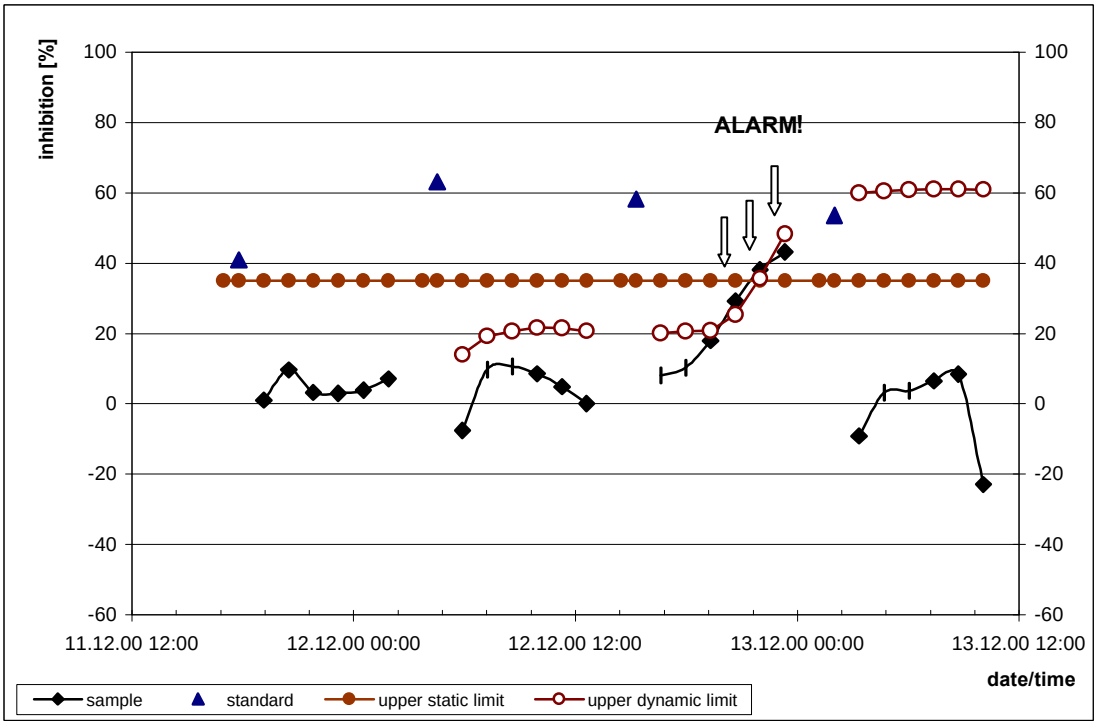

Fig. 6 Example of a monitoring of leachate

The lifespan of the immobilized bacteria amounted during these investigations from 2 to 4 days normally. With permanently high stress caused by pollutants the lifespan of the light-bacteria is possibly shorter than under normal conditions.

The IUT – Institut für Umwelttoxikologie provides the lyophilized „light-bacteria chips“ in cuvettes to the users. That includes a quality control (f.i. light intensity [minimum 10 V], inhibition values for Cr^{VI}, NaCl or 3,5-dichlorophenol in the range between 20 to 80 %). All other needed solutions such as nutrient solution, buffers, standard and reconstitution solutions are also provided by the IUT.

During the first measuring cycle alarm would be trigger off on the basis of only static threshold value. The static threshold value was calculated on the basis of the zero line of the *onlineTOX*® ± threefold

standard deviation. The minimum static threshold value is approximately 35 % inhibition of the luminescence of the light bacteria.
From the beginning of the second measuring cycle the dynamic threshold value is calculated additionally on the basis of the sliding median of six or twelve previous measurements ± threefold standard deviation.
During the third cycle we got three alarm signals on the basis of static and dynamic threshold values (see arrows in fig. 6) and at each alarm a retaining sample was collected.

Possible applications of the toxicity analyzer *onlineTOX*®

As a matter of principle the analyzer can be installed everywhere potential pollutants are produced or set free. The analyser is suitable for toxicity monitoring of watery environmental media. Examples of applications for an environmental early warning system are:

- toxicity monitoring of surface waters
- surveillance of parts of sewage before sewage treatment plants with biological purification
- surveillance of the total water stream before, respectively after central biological treatment
- surveillance of industrial dischargers
- toxicity monitoring of leachate drainage
- control of raw water pretreatment for drinking water winning
- surveillance of facilities that are working with water endangering materials
- control of drinking water processing

Summary

The toxicity analyser *onlineTOX*® determines the resulting toxic effects of environmental samples. By measuring the biological effect on a living microorganism most of chemical pollutants contained in the sample are taken in consideration – including toxic by-products.
By measuring the resulting overall effect all toxic combinations or exponential effects are observed which can usually determined by chemical analysis. Consequently, toxicologically justified threshold values can be set to trigger an alarm as soon as a determined hazardous potential is exceeded.
The user can enter a static and/or dynamic threshold value. The static threshold value is constant during the whole measuring time after starting the measuring program. On the other side the dynamic threshold value is calculated by the integrated microprocessor from the beginning of the second measuring cycle. It results from all previous sample inhibition values and allows consider for fluctuations or trends in the occurrence of pollutions.
The program-storage of the apparatus includes different measuring and service programs (f.i. rinsing programs). Also user specific solutions are possible.

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