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Review article

Toxicity assessment using different bioassays and microbial biosensors

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ABSTRACT

Toxicity assessment of water streams, wastewater, and contaminated sediments, is a very important part of environmental pollution monitoring. Evaluation of biological effects using a rapid, sensitive and cost effective method can indicate specific information on ecotoxicity assessment. Recently, different biological assays for toxicity assessment based on higher and lower organisms such as fish, invertebrates, plants and algal cells, and microbial bioassays have been used. This review focuses on microbial biosensors as an analytical device for environmental, food, and biomedical applications. Different techniques which are commonly used in microbial biosensing include amperometry, potentiometry, conductometry, voltammetry, microbial fuel cells, fluorescence, bioluminescence, and colorimetry. Examples of the use of different microbial biosensors in assessing a variety of environments are summarized.

Contents

Abbreviation: GC, gas chromatography; HPLC, high performance liquid chromatography,; AAS, atomic absorption spectroscopy.; LC, lethal concentration; ATP, adenosine triphosphate; EC_{50}, the effective concentration; NOEC, no observed effect concentration; MFC, microbial fuel cell; BOD, biological oxygen demand; BTE, benzene, toluene, ethylbenzene; IDE, interdigitated electrode.; SOB, sulfur-oxidizing bacteria.; Ppb, part per pillion; HRT, hydraulic retention time; EC, electrical conductivity; EDC, endocrine disrupting compounds; *Gfp*, green fluorescent protein; *PSII*, photosystem II; FMNH2, flavin mononucleotide; RCHO, long-chain fatty aldehyde; BTEX, benzene, toluene, ethyl benzene, and xylene.

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1. Introduction

Intensive industrialization and the use of chemicals in agriculture have contributed to the release of many toxic compounds into water, air, and soil, which causes many environmental problems (Jaffrezic-Renault and Dzyadevych, 2008). The exposure of living organisms to toxic levels of pollutants can cause disease to human and animals. Toxic chemicals can modify the rates of natural biological processes which include the long-term inhibition of growth, reproduction, and migration of species. Thus, the monitoring and detection of toxic chemicals is very important for the overall safety and security of humans and all biota on earth.

The seriousness of environmental pollution has brought forth a growing number of initiatives and scientific activity to assess water, air, and soil pollution. The analysis of toxic chemicals in environmental samples can be divided into two groups. In the first group, the pollutants are identified and quantified based on chemical or physical analyses such as gas chromatography (GC), high performance liquid chromatography (HPLC), or atomic absorption spectroscopy (AAS). In spite of their high sensitivity and accuracy in the determination of the concentration of pollutants in environmental samples, they have many disadvantages. For example, these techniques are time consuming because of the need for sample preparation and pre-concentration. They are also expensive and cannot be performed easily outside the laboratory. These methods enable the detection of a single compound or a limited group of chemicals at any given time. However, they do not give an indication of the cumulative toxicity of multiple contaminants in a sample which is of primary importance. In addition, all of these techniques require skilled personnel, expensive equipment, and it may take up to a few weeks to obtain results from these tests. The second group includes bioassays and biosensors. Here, the toxic chemicals are not clearly identified, but the measurements allow for the assessment of toxicity of environmental samples toward target organisms. These techniques are very helpful for assessing the risk associated with contaminated water samples. They rely on changes in the physiological response of living organisms which can be inferred on higher organisms and have many advantages such as rapid response, simplicity, specificity, sensitivity, and cost effectiveness.

2. Toxicity tests based on bioassays

Bioassays can be used for monitoring pollutants that cannot be easily detected by the restricted range of traditional methods. Bioassays have been used to establish toxicity toward both eukaryotes and prokaryotes exposed to different contaminants relative to a control where no toxic chemicals are present. Since toxic chemicals exert a cumulative and synergistic effect on the growth of organisms, bioassays can provide a clear and appropriate measure of toxicity of mixtures of toxins compared to traditional methods. In addition, the response of any analysis method that is specific to a single toxic chemical is insufficient to measure an adverse biological impact of a mixture of toxic chemicals to a generally diverse receiving ecosystem. Thus, bioassays that measure toxicity, in general, to a mixture of chemicals are needed.

Table 1 summarizes the most commonly used organisms to assess toxicity in water and wastewater. Test organisms include fish, invertebrates, plants, algae, and microorganisms. Some of these systems, e.g. animals and fish larvae, are difficult to handle and they do not provide a rapid response. Also, the use of some of these organisms may be ethically objectionable. Other systems, such as mammalian cells are expensive and results are not always consistent (Su et al., 2011; Tothill and Turner, 1996b).

Table 1
Bioassay tests.

	Organisms	Signal
Fish	Zebra fish embryos	Larval growth and survival
	Fathead minnow	Mortality, motility
	Bluegill	Cell division and differentiation
	Rainbow trout	ATP levels
	Salmonids	
Invertebrate	*Daphnia magna*	Mortality, motility
	Brachionus calyciflorus sp.	Viability and growth
	B. plicatilis	Enzymatic activity
	Artemia salina sp.	Number of daphnids
	Shrimps	Speed variation
	Bivalves, mussels	Open/closing of shell
Plants	Chinese cabbage	Micronucleus production,
	Oats	genotoxicity
	Vicia faba	Germination rate, biomass
	Allium cepa	weight, enzymatic activity
Algae	*Chlamydomonas* sp.	Mortality
	Chlorella sp.	Photosynthesis activity
	Chlorella vulgaris	Algal growth
	Monoraphidium sp.	Enzymatic activity
	Scenedesmus subspicatus	Cell counts
Bacterial	Nitrifying bacteria	Capacity of microorganisms to
	Nitrobacter	transform C, N, S, enzymatic activity,
	Nitrosomonas	microbial growth
	Bioluminescent bacteria	Mortality, photosynthesis activity
	Microbial fuel cells	Glucose uptake activity
	Activated sludge	Luminescence output
	Oligotrophic bacteria	Respiration, electrical output
	Aerobic bacteria	

2.1. Fish bioassays

Fish bioassays have been employed in toxicity assessment for many decades. The use of fish species as bioindicators in water is based on the assumption that fish species are sensitive indicators, which can detect the changes in the environment (Ziglio et al., 2008).

Fish show distinct physiological and behavioral responses toward pollutants. The fish bioassay is usually based on larval growth and survival where newly hatched fish are exposed to a range of effluents for 1–2 days or up to 7 days. The acute lethality test measures the lethal concentration (LC) of a chemical that is lethal to 50% of the exposed population after 96 h. Fish assays based on the measurement of adenosine triphosphate (ATP), the biochemical indicator of energy, in white muscle tissue are also carried out (Couture et al., 1989; Tothill and Turner, 1996b). Species such as zebra fish (*Brachydanio rerio*), fathead minnow (*Pimephales promelas*), bluegill (*Lepomis macrochirus*), guppy (*Poecilia reticulata*), rainbow trout (*Oncorhynchus mykiss*, *Salmo gairdneri*), red killifish (*Oryzias latipes*), common carp (*Cyprinus carpio*), and golden orfe (*Leuciscus idus*) are commonly used for acute lethality tests (Munkittrick et al., 1991). In addition, salmonids (e.g., *S. gairdneri*) are used for various assays to assess the toxicity of wastewater (Couture et al., 1989). Many types of fish are compatible and are the ultimate online toxicity assessment for stream water (Polak et al., 1996). They evaluate the total toxic effect of chemicals such as herbicides, heavy metals, and organic pollutants.

The advantages of using fish as a bioassay are that fish are present in all aquatic ecosystems and are at the top of the food chain which depends on many biotic factors. Most fish species are easily identified and are well suited for ecotoxicological assessment. In addition, fish are useful indicators of chronic toxicity as they have long life cycles. The main disadvantages of using fish as bioindicators are low sensitivity to low concentrations of toxic chemicals, long test periods, and specialized equipment and operators with adequate skills are needed (Farré and Barceló, 2003).

2.2. Invertebrate bioassays

Invertebrates are widely used in toxicity assessment of water ecosystems. The most common invertebrate used to characterize toxicity of water and wastewater treatment effluents is the water flea, *Daphnia magna*. Acute lethality tests with *D. magna* are well established and standardized (ISO, 2007; USEPA, 2002). The use of daphnids has many advantages for routine toxicity testing, such as high sensitivity to