Environment International 92–93 (2016) 106–118

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Review article

Toxicity assessment using different bioassays and microbial biosensors

Sedky H.A. Hassan [a], Steven W. Van Ginkel [b], Mohamed A.M. Hussein [c], Romany Abskharon [d], Sang-Eun Oh [e,*]

[a] Botany Department, Faculty of Science, Assiut University, New Valley Branch, 72511 Al-Kharja, Egypt
[b] School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA
[c] Assiut University Mycological Centre (AUMC), Assiut, Egypt
[d] National Institute of Oceanography and Fisheries (NIFO), 11516 Cairo, Egypt
[e] Department of Biological Environment, Kangwon National University, 200-701 Chuncheon, Kangwon-do, South Korea

ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form 5 March 2016
Accepted 5 March 2016
Available online 9 April 2016

Keywords:
Bioassay
Toxicity assessment
Microbial biosensors
Environmental pollution

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.

* Corresponding author: Department of Biological Environment, Kangwon National University, 200-701 Chuncheon, Kangwon-do, South Korea.
E-mail address: ohsangeun@kangwon.ac.kr (S.-E. Oh).

http://dx.doi.org/10.1016/j.envint.2016.03.003
0160-4120/

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

toxicants, simplicity, short exposure duration, short reproductive cycle, and parthenogenetic reproduction (Tothill and Turner, 1996b).

Living and motile daphnids are counted after a period of incubation with target contaminants. Acute (24 and 48 h) and chronic (21 days) toxicity tests with daphnids have been reported (Tišler et al., 2004). The test is conducted in small beakers or standard test tubes and often incorporates replication (e.g., 5 organisms in each of 4 replicates, 10 organisms in each of 2 replicates, along with basic water quality monitoring; i.e., temperature, dissolved oxygen, pH, hardness, and conductivity). The measurement endpoints evaluated are the 48-hour EC_{50} (the effective concentration (EC) where 50% of the population is immobile). Test organisms are assessed for the two assessment endpoints after each 24-hour interval. Daphnids are widely used as a test organism in acute and chronic toxicity tests of chemicals and complex effluents (Liu et al., 2002; Maltby, 2007). *D. magna* have been used successfully to predict the toxic effects of many pollutants in laboratory and field studies (Barata et al., 2006; Barata et al., 2007). Pretti et al. (2009), studied the acute toxicities of 18 ionic liquids using *D. magna*, and showed higher toxicity to *D. magna*.

The freshwater rotifer *Brachionus calyciflorus* and its marine counterpart *Brachionus plicatilis* have been used in toxicity tests (Janssen et al., 1994; Snell et al., 1991). Rotifers produce cysts (resting eggs) which can be stored for months and used on demand. Toxicity tests are conducted in sterile, 24-well, polystyrene plates. The test is initiated by introducing one neonate into each well containing food and test solution, followed by incubation of the plates at 25 °C in the dark. Survival and reproduction data are obtained by exposing isolated rotifer neonates to the toxicant and recording life history characteristics at regular intervals.

Additionally, other bioassays have been developed such as those based on the brine shrimp, *Artemia salina*. *A. salina* serves as the test organism in a wide range of toxicological assays and research; it was used in the screening of bioactive compounds in natural products, detection of cyanobacterial and algal toxicity in water, and detection of anthropogenic chemicals in the environment (Ruebhart et al., 2008). The *A. salina* bioassay is attractive for different reasons including (i) the commercial availability of the cysts, (ii) it can be maintained indefinitely in the laboratory in their cyst form and is easily induced to hatch, (iii) the assay is quick, simple, and performed at low cost, (iv) it requires small sample volume and can be performed with high sample throughput (microplates), and (v) it complies with animal ethics guidelines in many countries (Rosenfeldt et al., 2007).

Other toxicity assays using freshwater invertebrates as indicator organisms are based on survival and/or growth of mayflies, amphipods, stoneflies, or marine species, such as mysids, shrimps, or oysters (Price et al., 1990; Sarakinos et al., 2000; Suter Ii and Rosen, 1988). Assays are usually based upon results of visual or microscopic examination (Tothill and Turner, 1996b).With respect to labor demand and general requirements, tests with invertebrates are both practical and feasible. However, operator skills are required to culture and maintain test organisms.

2.3. Algal bioassays

Because of their ecological importance and sensitivity to many substances, especially herbicides and metals, algae are often used in toxicity testing. The test method described below has been widely used for many years to determine the toxicity of test materials to various species of microalgae.

Organisms of a particular species of microalgae are maintained under static conditions in test vessels containing nutrient medium alone (controls) and nutrient medium to which the test material is added. In preparation for the test, appropriate volumes of nutrient medium and/or test solution are placed in the test vessels (Erlenmeyer flasks) with replicates for each treatment. Algae are then introduced into the flasks, which are subsequently placed in a growth chamber which provides standardized light and temperature conditions. Each test vessel is inoculated at an initial population density to provide for growth sufficient to allow accurate quantification without resulting in nutrient or carbon dioxide limitation under the test conditions. Data on population growth, such as increases in dry weight or optical density, during the test are obtained on a daily basis for 96 h. The results of the test are expressed as the 96-h IC50 which is the inhibitory concentration (IC) where growth is reduced by half, based upon final population density and the average specific growth rate. The NOEC (no observed effect concentration) is also determined. Unlike scaled-down test methods, the flask method employs enough test solution for most chemical analytical procedures. The most common algal species used are *Chlamydomonas variabilis*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Chlorella kessleri*, *Monoraphidium pusillum*, *Scenedesmus quadricauda*, *S. subspicatus*, and *Selenastrum capricornutum*.

Assessment of endpoints using microalgae for acute and chronic tests includes esterase inhibition, ATP energy loss, growth inhibition, motility inhibition, and chlorophyll fluorescence. Many algal bioassays are used to assess the toxicity of organic pollutants, herbicides, oil dispersants, effluents, solid waste leachates, groundwater, and organic extracts.

2.4. Plant bioassays

Plant bioassays, such as oats (*Avena sativa*) and Chinese cabbage (*Brassica campestris*) offer several advantages such as a large array of assessment endpoints (germination rate, biomass weight, enzyme activity, etc.), low maintenance cost, rapid test activation, and the special advantage of being able to assess the toxicity of solid wastes (Ferrari et al., 1999). Tests based on growth responses of the plant are potentially sensitive but require a long time for growth to occur, e.g., 4–6 days for length measurements of the roots and shoots, 14–30 days for a fresh or dry weight measurement, and 21 days for germination scores (Ou et al., 1994).

2.5. Microbial bioassays

Many of the previous tests such as fish, invertebrate, and plant bioassays require specialized equipment and operator skills, long acclimatization times, and are labor intensive, expensive, and time consuming. The use of higher organisms may also be ethically undesirable. The need to have indicator systems which can rapidly assess the toxicity of effluents has led to research into toxicity tests which are rapid, easy, and inexpensive. In the last few years, there has been increased interest in microbial screening tests for assessing toxicity. Microbial bioassays for toxicity assessment offer some advantages; they are inexpensive, have short life cycles, are less time consuming, and are sensitive to different toxic chemicals such as heavy metals, phenolic compounds, organic pollutants, endocrine disruptors, and effluents from industrial activities (Lei et al., 2006b; Su et al., 2011; Tothill and Turner, 1996b). In addition, most microorganisms have similar biochemical pathways as higher organisms and respond rapidly to environmental changes.

Wide varieties of microbial techniques have been developed and are used as toxicity screening procedures. Microbial bioassays use different mechanisms based on (i) the capacity of microorganisms to transform carbon, sulfur, or nitrogen, (ii) enzymatic activity, such as the utilization of ATP, acid and alkaline phosphatase, dehydrogenases, and other enzymes (iii) microbial growth, mortality, or photosynthetic activity, (iv) glucose uptake, (v) oxygen consumption, and (vi) luminescence output (Tothill and Turner, 1996a). Bioassays based on dehydrogenases use specific dyes such as methylene blue, triphenyltetrazolium chloride, and resazurin which can be used as electron acceptors, changing color once they are in reduced form (Tothill and Turner, 1996b). ATP, the keystone of all cellular activity, is present in all living cells and is rapidly destroyed upon the death of organisms. The basis of the ATP toxicity assay is based on the change in ATP content in the presence of toxic chemicals. The method of ATP detection is based on the light producing

reaction of the luciferin-luciferase enzyme system derived from the firefly in which light production is proportional to the amount of ATP present (Dalzell and Christofi, 2002).

The activated sludge respiration inhibition test has been established as an effective method for evaluating the toxicity of chemicals to activated sludge (ISO, 2007; OECD, 2014). The most common measure of respiration is the oxygen uptake rate. The respiration rates of samples of activated sludge fed with synthetic sewage are measured in an enclosed cell containing an oxygen electrode after a contact time of 3 h (OECD, 2014). The most common measure of the bacterial respiration rate is the oxygen uptake rate. Liao et al. (2001) described a biosensor for wastewater toxicity based on the inhibition of the respiration of oxygen sensitive bacteria (strain unspecified) isolated from activated sludge. When the respiration of the bacteria was inhibited due to toxicity, more oxygen was able to cross the membrane in the biosensor, leading to a change in the rate of the oxidation–reduction reactions that occurred on the membrane. The response time of the biosensor was shown to be approximately 8 min. dos Santos et al. (2002) developed an activated sludge respirometry-based assay using 96-well microplates. In this assay, respiration was indirectly quantified. In this case, a dye (tetrazolium violet) was mixed with sludge bacteria in the wells of the microplate. The dye was chemically reduced when bacterial respiration occurred, yielding a deep purple color. In this way, the color of the dye was used as an indicator of sludge respiration. An incubation time of 24 h was needed and may limit the usefulness of this assay for influent toxicity monitoring, which requires a quick response. A similar assay was later used to evaluate nitrification inhibiting substances in industrial wastewaters (Svenson et al., 2000). The consumption rates of ammonium-nitrogen and nitrite-nitrogen relative to controls were used to calculate nitrification inhibition levels in *Nitrosomonas* and *Nitrobacter* assays, respectively.

Luminescent microorganisms have been used in the production of several toxicity test instruments. The bioluminescence inhibition assay is based on marine gram negative bacteria, *Vibrio fischeri* or *Photobacterium phosphoreum.* The specific strain, *V. fischeri* NRRL B-11177, has been widely used for acute toxicity estimation and several commercial test kits, i.e., Microtox, LUMIStox, and ToxAlert are based on this strain (Shijin, 2004). The test relies on the change in the bacterial luminescence when the microorganisms are exposed to toxic chemicals. The bioluminescence inhibition of *V. fischeri* has been standardized (OECD, 2014) and the test is commercially available in different versions. The advantages of these toxicity tests include short analysis times and simplicity of operation.

3. Biosensors

Biosensors are analytical devices, constructed by combining a biological sensing element (e.g., enzymes, antibodies, microorganisms, or DNA) with a transducer (e.g., electrochemical, optical, colorimetric, or piezoelectric transducers) to obtain a useable signal output (Bentley et al., 2001; D'Souza, 2001; Dennison and Turner, 1995; Lei et al., 2006a; Marazuela and Moreno-Bondi, 2002; Su et al., 2011; Zhai et al., 1997). A whole-cell biosensor contains living organisms, such as bacteria, microalgae, yeast, or fungi as the sensing element and a method of integrating general metabolic status via the transducer. The signal output can be a change in proton concentration, release or uptake of gases, light emission, or absorption. The transducer converts the biological signal into a measurable response such as current, potential, or absorption of light through electrochemical or optical means, which can be further amplified, processed and stored for later analysis (Lei et al., 2006a).

While classical analytical methods usually involve laborious work and sample pretreatment, biosensors offers some advantages in identifying and quantifying specific compounds directly in air or water. Furthermore, while classical analytical methods usually provide a measurement of the total concentration of potentially toxic chemicals, they don't provide a true assessment of bioavailability or their toxic impact toward living organisms (Rodriguez-Mozaz et al., 2005). Biosensors can complement classical analytical methods because they are able to distinguish between bioavailable and unavailable forms of contaminants.

Biosensors are usually classified into various basic groups according to the method of signal transduction. Accordingly, biosensors can be categorized as electrochemical, optical, piezoelectric, or thermal based on the transducing element; and as immunochemical, enzymatic, DNA-based, or whole-cell based on the means of biorecognition. Different groups of biosensors, including optical (Homola et al., 1999; Marazuela and Moreno-Bondi, 2002), electrochemical (Thévenot et al., 2001) thermal (Ramanathan and Danielsson, 2001) micro electro mechanical (Moulin et al., 2000), immune-based (Hock, 1997), whole-cell (Bousse, 1996) and DNA-based (Collings and Caruso, 1997) were discussed in detail in the literature. Table 2 shows different types of microbial biosensors and their transducers which are used to assess different toxic chemicals in the environment.

The ideal biosensor has to have two main properties: sensitivity (response to low concentrations of analytes) and specificity (ability to discriminate among analytes according to the bio-recognition elements) (Eltzov and Marks, 2011). The sensitivity of biosensors depends not only on the chemical complexity of the sample, i.e., the type and quantity of analytes present, but also on the physiological state of the cells, enzymes, or DNA at the time of measurement.

In order to effectively transform the biochemical response into a physical signal, the microbial cell's “recognition element” must be immobilized to the transducer. Therefore, immobilizing microorganisms on transducers plays an important role in the fabrication of microbial biosensors (D'Souza, 2001; Lei et al., 2006a; Su et al., 2011). Traditional methods for the immobilization of microorganisms include

Table 2
Microbial biosensors.

Biosensor type	Transducer	Advantages	Disadvantages
Optical	Bioluminescence Fluorescence Colorimetric Absorption Reflection	Simplicity, flexibility, multichannel sensing, remote sensing, electrically passive, wide dynamic range	Light interference, need exists for more selective indicators and more immobilization steps
Electrochemical	Amperometric Potentiometry Voltammetry Impedimetric Microbial fuel cell Conductivity	Sensitive, compatible with modern microfabrication technologies, portability, low cost, requires minimal power	Interferences related to electrochemical reaction of species other than other analyte
Thermal	Heat changes Calorimetry	Multichannel sensing, high stability, continuous monitoring	Lack of specificity, Application mainly in medical application
Mass sensitive	Piezoelectric	Real-time output Simplicity	Less sensitivity

adsorption, encapsulation, entrapment, covalent binding, and cross-linking (Lei et al., 2006a; Su et al., 2011). Physical adsorption is the simplest method for microbe immobilization. In this method, a microbial suspension is incubated with the electrode or an immobilization matrix, followed by rinsing with buffer to remove un-immobilized cells. The microbial cells are immobilized due to adsorptive interactions such as ionic, polar or hydrogen bonding, and hydrophobic interactions (Lei et al., 2006a).

4. Biosensors classification

Biosensors are usually classified either according to the transduction element (electrochemical, optical, mass sensitive, piezoelectric or thermal) or to the bioreceptor (enzymatic, immune affinity recognition, whole-cell, or DNA). Electrochemical and optical techniques are most widely used in the development of microbial biosensors.

4.1. Electrochemical microbial biosensors

Electrochemical approaches are widely used in the development of microbial biosensors and can be divided into amperometry, potentiometry, conductometry, voltammetry, and microbial fuel cell (MFC) (Su et al., 2011). Electrochemical biosensors offer high sensitivity, compatibility with modern micro-fabrication technologies, portability, low cost, and minimal power requirement (Wang, 2002).

Amperometry is based on the changes in the applied potential between the working electrode and the reference electrode and the current signal is recorded and correlated with the concentration of target compounds. In the amperometric detection, the current signal is generated due to the reduction or oxidation of an electro-active metabolic product or intermediate on the surface of a working electrode (Ding et al., 2008; Su et al., 2011).

Conductometry is a technique depending on the conductivity change in the solution due to the production or consumption of ionic species; for example, by the metabolic activity of the microorganisms (Lei et al., 2006a; Rodriguez-Mozaz et al., 2005; Su et al., 2011; Wang, 2002). The measurement of conductance is extremely fast and sensitive compared to sophisticated modern analytical techniques, making conductometric microbial biosensors very attractive (Su et al., 2011). It is also worth noting that such biosensors are suitable for miniaturization since it requires no reference electrode in the system (Shul'ga et al., 1994). However, all ions that pass through the biosensor change the conductivity, thus the selectivity of conductometric biosensors is relatively poor (Mikkelsen and Rechnitz, 1989).

Potentiometry is based on the monitoring of the potential difference between the working electrode and the reference electrode and the potential signal exhibits concentration-dependent behavior. The transducers employed in the potentiometric techniques are usually a gas-sensing electrode or an ion-selective electrode such as pH, ammonium, or chloride (Bobacka et al., 2008; Lei et al., 2006a; Su et al., 2011). The sensitivity and selectivity of potentiometric biosensors are outstanding due to the species-selective working electrodes used in these systems. However, a highly stable and accurate reference electrode is always required and challenging to maintain, which may potentially limit the application of potentiometry in microbial biosensors (Su et al., 2011).

Voltammetry is the most versatile technique in electrochemical analysis. Both the current and the potential are measured and recorded (Bobacka et al., 2008; Su et al., 2011). The position of peak current is related to the specific chemical and the peak current density is proportional to the concentration of the corresponding species. A remarkable advantage of voltammetry is the low noise which can endow the biosensor with higher sensitivity (Bobacka et al., 2008). In addition, voltammetry is able to detect multiple compounds, which have different peak potentials, in a single electrochemical experiment (or scan), thus offering the simultaneous detection of multiple analytes. Furthermore, an effective pre-concentration step (electrochemical stripping analysis) makes the voltammetric technique one of the most sensitive electroanalytical methods (Wang, 2002).

Microbial fuel cells (MFCs) convert chemical energy into electrical energy by means of the metabolic activity of microorganisms (Hassan et al., 2014; Hassan et al., 2012; Logan, 2009). Since the consumption of target compounds by microbes or the inhibition of the metabolic pathway(s) by toxic compounds can potentially alter the production of electricity, MFCs can be applied as a microbial biosensor for *in situ* analysis and for monitoring target chemicals. A change in electrical current indicates a change in water quality. Recently, MFCs have been used for the detection of acetate. *Geobacter sulfurreducens* was used and the current generated was correlated to varying concentrations of acetate in the influent (Tront et al., 2008). Stein et al. (2010) used MFCs to detect toxic chemicals such as copper and found that changes in current density were correlated to changes in copper toxicity.

4.1.1. Amperometric microbial biosensors

Amperometry is the most widely used technique in electrochemical microbial biosensors. Table 3 summarizes recently reported amperometric microbial biosensors in the literature. Amperometric microbial biosensors have been extensively exploited for environmental applications including the measurement of biological oxygen demand (BOD) (Chang et al., 2004; Karube et al., 1977; Liu et al., 2000; Liu and Mattiasson, 2002).

Several amperometric BOD biosensors are based on an oxygen electrode transducer modified with microorganisms which degrade organic pollutants (Chan et al., 2000; Karube et al., 1977; Lei et al., 2006a; Liu et al., 2000; Liu and Mattiasson, 2002; Su et al., 2011). The microbial strains used include *Torulopsis candida* and *Trichosporon cutaneum* (Sangeetha et al., 1996), *Pseudomonas putida* (Chee et al., 1999), *Klebsiella oxytoca* AS1 (Ohki et al., 1994), *Bacillus subtilis* (Tan and Qian, 1997), *Arxula adeninivorans* LS3 (Chan et al., 2000; Chan et al., 1999; Tag et al., 2000), *Serratia marcescens* LSY4 (Kim and Kwon, 1999), *Pseudomonas* sp. (Li and Chu, 1991), *P. fluorescens* (Yoshida et al., 2000), *P. putida* SG10 (Chee et al., 2005), thermophilic bacteria (Karube et al., 1989), and yeast (Kulys and Kadziauskiene, 1980).

Phenol and substituted phenols have received considerable attention in waste analysis programs due to their high toxicity to living organisms including humans, animals, and plants. A variety of amperometric microbial biosensors have been reported for these EPA Priority chemicals (Lei et al., 2006a; Lei et al., 2003, 2004; Mulchandani et al., 2005; Mulchandani et al., 2002; Timur et al., 2003; Timur et al., 2004).

Other microbial biosensors for phenols include a *Rhodococcus erythropolis* modified Clark oxygen electrode for 2,4-dinitrophenol (2,4-DNP) detection (Emelyanova and Reshetilov, 2002) and *P. putida* DSM 50026, a well-known phenol degrading microorganisms, used to modify screen printed graphite electrodes for phenol detection (Timur et al., 2003; Timur et al., 2004).

The inhibition of bacterial respiration and hence the decrease of oxygen consumption rate, has been utilized to fabricate a cyanide biosensor (Ikebukuro et al., 1996a; Okochi et al., 2004). Whole-cell biosensors consisting of a dissolved oxygen electrode modified with *Nitrosomonas europaea*, *Thiobacillus ferrooxidans*, *Saccharomyces cerevisiae*, and *Pseudomonas fluorescens* were reported for batch and continuous cyanide monitoring (Ikebukuro et al., 1996a; Lee and Karube, 1996; Nakanishi et al., 1996; Okochi et al., 2004). Based on the respiratory activity of the bacteria, target analyte adapted *P. putida* has been used as the sensing element in the detection of pollutants, such as BTE (benzene, toluene, ethylbenzene) (Rasinger et al., 2005) and 2,4-dichloro phenoxyacetic acid (Odaci et al., 2009).

In addition, amperometric microbial biosensors also provide rapid and sensitive tools in health and fermentation applications. For example, the detection of glucose, which is of great interest in the treatment

Table 3
Amperometric microbial biosensors.

Target	Microorganisms	Detection limit	References
BOD	*Arxula adeninivorans* LS3	1.24 mg/L	Chan et al. (2000)
BOD	*Candida parapsilosis*	1 mg/L	König et al. (2000)
BOD	*Trichosporon cutaneum* and *B. subtilis*	0.5 mg/L	Jia et al. (2003)
BOD	*Torulopsis candida*	7–75 mg/L	Sangeetha et al. (1996)
BOD	*Trichosporon cutaneum*	0–32 mg/L	Yang et al. (1997)
BOD	*Trichosporon cutaneum*	10–70 mg/L	Marty et al. (1997)
BOD	*P. putida*	0–0.5 mg/L	Chee et al. (1999)
BOD	*B. subtilis*	2–22 mg/L	Riedel et al. (1988)
BOD	*A. adeninivorans* LS3	8–550 mg/L	Riedel et al. (1998)
BOD	*Serratia marcescens* LSY4	0–44 mg/L	Kim and Kwon (1999)
BOD	*Pseudomonas* sp.	1–40 mg/L	Li and Chu (1991)
BOD	*P. fluorescens*	15–200 mg/L	Yoshida et al. (2000)
BOD	*P. fluorescens*	15–260 mg/L	Yoshida et al. (2001)
BOD	*P. putida* SG10	1 mg/L	Chee et al. (2005)
BOD	*T. bacteria*	<10 mg/L	Karube et al. (1989)
BOD	*B. subtilis* and *B. licheniformis* 7B	10–70 mg/L	Tan et al. (1992)
BOD	*B. subtilis* and *B. licheniformis* 7B	0–80 mg/L	Tan et al. (1993)
BOD	Yeast SPT1 and SPT2	2 mg/L	Rastogi et al. (2003)
p-Nitrophenol	*Arthrobacter* sp. JS	0.027 mg/L	Lei et al. (2003)
p-Nitrophenol	*Moraxella* sp.	0.014 mg/L	Mulchandani et al. (2005)
2,4-Dinitrophenol	*Rhodococcus erythropolis*	0.36–7.3 mg/L	Emelyanova and Reshetilov (2002)
Phenolic compounds	*P. putida*	0.047–0.56 mg/L	Timur et al. (2003)
Phenolic compounds	*P. putida*	0.01–0.1 mg/L	Timur et al. (2004)
Cyanide	*S. cerevisiae*	0.004 mg/L	Ikebukuro et al. (1996a)
Cyanide	*T. ferrooxidans*	0.0135 mg/L	Okochi et al. (2004)
Cyanide	*P. fluorescents* NCIMB 11764	0.05–1 mg/l	Lee and Karube (1996)
Cyanide	*S. cerevisiae* IFO 0377	0–0.041 mg/L	Ikebukuro et al. (1996b)
Hydrogen peroxide	*Acetobacter peroxydans*	0.003–0.03 mg/L	Rajasekar et al. (2000)
Cu	Recombinant *S. cerevisiae*	31.7–127 mg/L	Lehmann et al. (2000)
Cd	Recombinant *E. coli*	2.8 mg/L	Biran et al. (2000)
Paraoxon	*P. putida* JS444	0.055 mg/L	Lei et al. (2005)
Paraoxon	*Moraxella* sp.	0.1 mg/L	Mulchandani et al. (2006)
Fenitrothion	*P. putida* JS444	0.227 mg/L	Lei et al. (2006b)
Phenol	*P. putida*	0.592–2.96 mg/L	Kirgoz et al. (2006)
Phenol	*E.coli*	1.6–16 mg/L	Neufeld et al. (2006)
Phenol	*P. putida*	37–296 mg/L	Timur et al. (2007a)
Benzene	*P. putida* F1	1.56–11.1 mg/L	Rasinger et al. (2005)
Toluene	*P. putida* F1	4.6–18.4 mg/L	Rasinger et al. (2005)
Ethylbenzene	*P. putida* F1	10.6–21.23 mg/L	Rasinger et al. (2005)
Catechol	*P. putida*	2.75–22 mg/L	Timur et al. (2007b)
Atrazine	*C. vulgaris*	0.21 mg/L	Shitanda et al. (2009)
Cu^{2+}	*S. cerevisiae* (I)	2.1 mg/L	Tag et al. (2007)
Fe^{2+}	*A. ferrooxidans*	0.05 mg/L	Zlatev et al. (2006a, 2006b)
Cr_2O_7	*A. ferrooxidans*	0.117 mg/L	Zlatev et al. (2006b)
2,4-Dichlorophenol	Mixed bacteria	3.27 mg/L	Jantra et al. (2005)
Cyanide	*Klebsiella* sp.	0.2 mg/L	Mak et al. (2005)
Anionic surfactants	*Pseudomonas* and *Achromobacter*	0.01 mg/L	Taranova et al. (2002)
Cadmium	*Escherichia coli*	0.6 mg/L	Biran et al. (2000)
(Paraxon)	*Flavobacterium*	2.75 mg/L	Gaberlein et al. (2000)
Atrazine	*C. vulgaris*	0.21 mg/L	Shitanda et al. (2005)
Benzene	*C. vulgaris*	121 mg/L	Shitanda et al. (2005)
Toluene	*C. vulgaris*	186.3 mg/L	Shitanda et al. (2005)

of diabetes and the quality control of fermentation, accounts for about 85% of the entire biosensor market (Wang, 2008).

4.1.2. The conductometric microbial biosensors

Conductometric microbial biosensors are based on the fact that almost all enzymatic reactions involve either consumption or production of charged species and, therefore, lead to a global change in the ionic composition of a tested sample (Lawrence and Moores, 1972). Biosensors based on the conductometric principle present a number of advantages: a) thin-film electrodes are suitable for miniaturization and large scale production using inexpensive technology, b) they do not require any reference electrode, c) transducers are not light sensitive, d) the driving voltage can be sufficiently low to reduce power consumption, and e) a large spectrum of compounds of different natures can be identified on the basis of various reactions and mechanisms (Jaffrezic-Renault and Dzyadevych, 2008).

The conductometric microbial biosensor is attractive and appealing owing to its fast and sensitive response to analytes. In this regard, a conductometric biosensor using *C. vulgaris* microalgae as the bioreceptor was constructed to detect heavy metal ions and pesticides in water samples (Chouteau et al., 2005). *C. vulgaris* was immobilized on a bovine serum albumin membrane deposited on a platinum interdigitated electrode (IDE). The developed biosensors were sensitive to Cd^{2+} and Zn^{2+} with a limit of detection of 10 ppb for both ions after a 30 min exposure. Pb^{2+} did not introduce any significant interference since it was likely adsorbed on albumin (Chouteau et al., 2005).

4.1.2.1. Sulfur oxidizing bacteria biosensors. Recently, a novel biosensor has been developed for detecting toxicity in water and stream water using sulfur-oxidizing bacteria (SOB). Sulfur-oxidizing bacteria are chemoautotrophic bacteria that oxidize inorganic sulfur (S°) compounds to

sulfuric acid (H_2SO_4) under aerobic conditions according to the following equation (Hassan et al., 2010; Oh et al., 2011).

$$S^\circ + H_2O + 1.5O_2 \rightarrow SO_4^{2-} + 2H^+ \Delta G^{\circ\prime} = -587.1 \text{ kJ/reaction}$$

The SOB biosensor relies on the production of hydrogen ions (H^+) which lowers the medium pH, and SO_4^{2-} which increases the electrical conductivity (EC) of the medium. The increase in EC and the decrease in pH indicate that SOB attach to S° particles and oxidize the S° to H_2SO_4 using O_2 as the electron acceptor. In the presence of toxic chemicals, the activity of SOB will be inhibited, which will cause an increase in pH and a decrease in EC (Gurung et al., 2011; Oh et al., 2011; Van Ginkel et al., 2011). Thus, toxicity can be detected using simple EC and pH meters (Hassan et al., 2010; Oh et al., 2011). A schematic diagram of sulfur oxidizing bacteria biosensor was shown in Fig. 1.

SOB biosensors can detect toxic metals including Cr^{6+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, and Zn^{2+}(Gurung et al., 2011; Hassan et al., 2010; Oh et al., 2011).The first study for toxicity assessment using the SOB biosensor was done by (Oh et al., 2011). In this study, the SOB biosensor was packed with 50 mL of S° particles (2–4 mm diameter) and air was introduced from the bottom at a flow rate of 150–250 mL/min. The biosensor was inoculated with sludge and incubated at 30 °C for 3 days in batch mode. The reactors were then fed with synthetic stream water, continuously in upflow mode using adjustable peristaltic pumps. The reactors were operated at a hydraulic retention time (HRT) of 30 min for 2–3 days to reach steady-state conditions (i.e. stable effluent EC and pH values) and then the toxic chemical, Cr^{6+} (2000 ppb), was spiked to the influents of the biosensors. The influent pH and EC averaged 7.1 and 0.12 mS/cm, respectively. The effluent EC and pH values were monitored every 10 min and data were saved on a computer using a data acquisition system. When 2000 ppb Cr^{6+} was added to the system, the effluent EC decreased from 1.15 mS/cm compared to the influent EC of 0.12 mS/cm in 5 h, while the pH increased from 2.1 to 6.5.

In the study by Hassan et al. (2010), they investigated the ability of SOB biosensors for the detection of other heavy metals including Zn^{2+}, Hg^{2+}, Cd^{2+}, and Pb^{2+}. The detection of Hg^{2+} (2 ppm), Cd^{2+} (2 ppm), and Pb^{2+} (2 ppm) was the greatest with an almost immediate decrease in EC. They observed that the percentage of inhibition was increased by increasing the exposure time or the concentration of the toxic chemical in the influent. When a mixture of heavy metals (Cd^{2+}, Hg^{2+}, Zn^{2+}, and Pb^{2+}) at 50 ppb each were added to the influent of the SOB biosensors at an HRT of 30 min, the effluent EC decreased rapidly due to the synergistic inhibitory effect of the heavy metals on SOB (Hassan et al., 2010).

The high sensitivity of SOB for the detection of heavy metals could be explained by the lower pH (2–3) of the biosensor. The lower pH likely makes metals more bioavailable and thus more able to assert their toxic effect compared to others biosensors which operate at a neutral pH.

SOB biosensors have the ability to monitor other pollutants such as endocrine disrupting compounds (EDC) in water. For example, Van Ginkel et al. (2010) studied the possibility of SOB biosensors for the detection of endocrine disrupting compounds including bisphenol-A, nonylphenol, estradiol, diethylstilbestrol, and tributyltin. When these chemicals were injected to the system, the effluent EC decreased and pH increased due to inhibition of SOB by the EDCs.

The SOB biosensor was also applied to assess the toxicity of a textile industry effluent and river water downstream from a textile plant in Korea (Gurung et al., 2011). In this study, SOB biosensors were operated in continuous mode using synthetic stream water at HRTs of 30 min until steady state (i.e. stable pH and EC values) and then the textile industry effluent or river samples were added to the biosensors and the effluent EC and pH values were monitored. They tested three samples –textile effluent (company 1 named as C1) and 2 river water samples (named as R1 and R2). When C1 was introduced to the influent, the effluent EC rapidly decreased from 1.83 mS/cm to the influent EC (1.25 mS/cm) in 1 h (Fig. 2a). The rapid decrease of EC in the effluent could be explained by the existing mixture of toxic chemicals present in the samples such as heavy metals (Pb^{2+}, Zn^{2+}, Hg^{2+},Cd^{2+}) and 1.4 dioxane. When C1 was diluted two times with the synthetic stream water and fed to the SOB biosensor, the EC decreased more slowly than when C1 was not diluted (Fig. 2b). The EC decreased and became stable at the influent EC (0.68 mS/cm^{-1}) after 1.5 h. C1 was then diluted 10 and 100 times with the synthetic stream water and fed to the SOB biosensors. In the case of 10× diluted C1, the EC decreased from 0.45 to 0.38 mS/cm^{-1} in 2 h which shows inhibition of the SOB (Fig. 2c). On the other hand, the 100× diluted C1 did not show any inhibition of the SOB (Fig. 2d). These results show that after dilution, the concentration of toxic chemicals is very low, and thus the toxicity of effluent diluted by stream water is reduced.

For R1, the EC gradually decreased within two hours after the addition of R1 to the biosensor. The EC decreased to a minimum in 7 h.

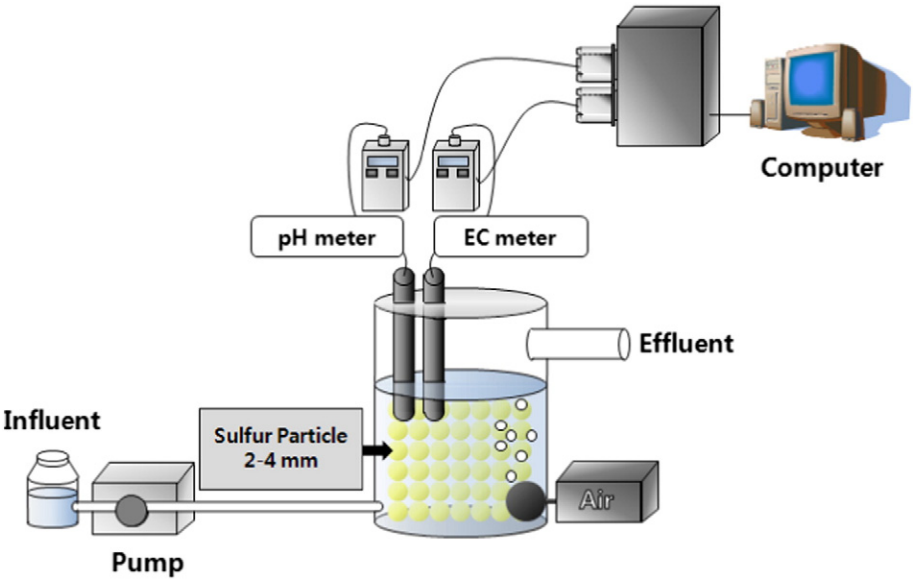

Fig. 1. A schematic diagram of sulfur oxidizing bacteria biosensor.
The figure is adapted from Hassan et al. (2010).

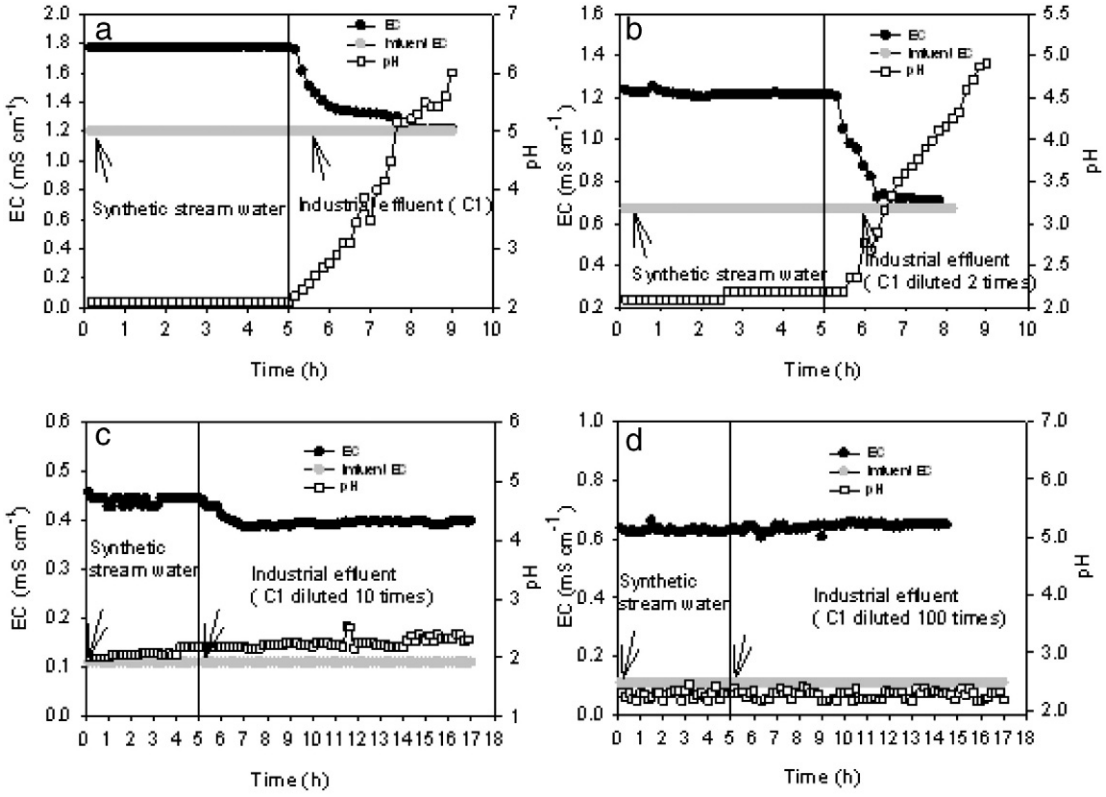

Fig. 2. Assessing textile effluent toxicity: a. (without dilution), b. (2 times diluted), c. (10 times diluted), and d. (100 times diluted). The figure is adapted from Gurung et al. (2011).

However, after 7–8 h of operation, the EC slightly increased and remained constant thereafter. Upon feeding R2, the EC changed instantaneously. The EC continued to decrease sharply for 2 h and then remained constant without showing any further decrease in EC. R2 appears to have been more toxic than R1 which can be explained by the higher concentrations of complex mixtures of toxic chemicals, such as heavy metals ions and oxidized chemicals such (nitrite), discharged from an industrial complex and wastewater treatment plants upstream of where the R2 sample was taken (Gurung et al., 2011).

In a recent study by Gurung et al. (2012), SOB biosensors were operated in semi-continuous mode for the detection of toxic chemicals in water. The SOB biosensors were fed with synthetic stream water (50 mL/min; 1 min rapid feeding, and 29 min batch reaction) in upflow mode using adjustable peristaltic pumps. After steady-state conditions (i.e. stable EC and pH values) were reached, Cr^{6+} (1 mg/L) was injected into the biosensor influents and effluent EC and pH values were monitored. The effluent EC decreased immediately after Cr^{6+} injection and after 5 h, complete inhibition (i.e. effluent EC and pH values equaled influent values) was observed due to Cr^{6+} toxicity to SOB. SOB activity recovered when Cr^{6+} was removed from the influent.

The use of the SOB-based biosensor has many advantages. First, toxicity can be measured using simple EC and pH meters, they use little energy, and they can be operated for at least several months. Second, periodic calibration of EC and pH and meters is not needed because EC or pH changes over time are more important than exact values (Oh et al., 2011). Third, sulfur particles are very cheap and no toxic byproducts are produced - the total mass of sulfate produced is low (Oh et al., 2011; Van Ginkel et al., 2011). Fourth, high sensitivity – contaminants can be detected in the low ppb range. The increased sensitivity is due to the low operating pH of the SOB biosensor (Van Ginkel et al., 2011). Under the acidic conditions of the SOB biosensor, nearly all of the toxic contaminants are in their free, protonated, or more soluble forms, which increases their bioavailability. In addition, at the low pH, maintenance requirements to maintain homeostasis is high; thus contamination by other microorganisms is avoided. Fifth, the detection of toxic chemicals increases with decreasing HRT which makes for rapid detection. Six, the SOB biosensor is active over a wide range of temperatures, the optimum temperature being 45 °C. Sixth, SOB biosensors can be operated in batch or continuous mode depending on the chemicals to be detected. Since the SOB biosensor gives a biological response to toxicity, the response to the additive and synergistic effect of multiple contaminants is likely a better predictor of overall toxicity than the measurement of individual chemicals.

4.1.3. Potentiometric microbial biosensors

Potentiometric microbial biosensors detect the amount of analytes by measuring the potential difference between a working electrode and a reference electrode separated by a selective membrane. Recently, a potentiometric biosensor based on a pH electrode modified by permeabilized *P. aeruginosa* was developed for the selective and rapid detection of the cephalosporin group of antibiotics (Kumar et al., 2008). The hydrolysis of cephalosporin, due to the enzyme activity of the microbial layer, was accompanied by the production of protons near the pH electrode. The response came from the change of the electric potential difference between the working electrode and the reference electrode (Kumar et al., 2008). Table 4 summarizes recently reported potentiometric microbial biosensors in the literature.

4.2. Optical microbial biosensors

Optics is another commonly used technique in microbial biosensors. The various types of optical transducers exploit properties such as simple light absorption, fluorescence/phosphorescence, bio/chemiluminescence, reflectance, Raman scattering, refractive index, or other optical signals produced by the interaction of microorganisms with the analytes, and correlates the observed optical signal with the concentration of target compounds (D'Souza, 2001; Lei et al., 2006a; Rodriguez-Mozaz et al., 2004; Su et al., 2011). Optical sensing

Table 4
Potentiometric microbial biosensors.

Target	Microorganisms	Detection limit	References
Cephalosporins	*P. aeruginosa*	41.5–4565 mg/L	Kumar et al. (2008)
Cephalosporins	*B. stearothermophilus* var	–	Ferrini et al. (2008)
Organophosphates	*Flavobacterium* sp.	8.1–30 mg/L	Gaberlein et al. (2000)
Organophosphates	Recombinant *E. coli*	0.65 mg/L	Mulchandani et al. (1998a, 1998b)
Organophosphates	Recombinant *E. coli*	0.97 mg/L	Mulchandani et al. (1998b)
Penicillin	Recombinant *E. coli*	1215–7290 mg/L	Galindo et al. (1990)
Urea	*Bacillus* sp.	0.036–33 mg/L	Verma and Singh, 2003
Trichloroethylene	*P. aeruginosa* JI104	0.1–4 mg/L	Han et al. (2002)
Ethanol	*S. ellipsoideus*	0.92–2300 mg/L	Rotariu et al. (2004)
Paraoxon	Recombinant *E. coli*	0.27–275 mg/L	Rainina et al. (1996)
Paraoxon	*E. coli* cells with surface expressed OPH	15.14–495.4 mg/L	Mulchandani et al. (1998a, 1998b)
Methyl parathion		26.3–212.6 mg/L	
Diazinon		152–2614 mg/L	

techniques are especially attractive in high throughput screening since they enable biosensors to monitor multiple analytes simultaneously (Brogan and Walt, 2005).

4.2.1. The fluorescent biosensor

The fluorescent sensing technique is based on the measurement of fluorescence intensity which is proportional to the concentration of the target analyte (Su et al., 2011). Fluorescent biosensors have been widely applied in analytical chemistry due to their easy construction using standard molecular biology techniques (Ibraheem and Campbell, 2010). Fluorescence-based microbial biosensors are divided into two categories: *in vivo* and *in vitro* types. In the *in vivo* fluorescent microbial biosensors, the microorganism(s) are able to produce a fluorescent substance, such as green fluorescent protein (*gfp*), without the addition of any fluorescent materials. For the in vitro type, the metabolites of microorganisms change the environment surrounding them, which lead to changes in light emissions as the fluorescent element is produced (Su et al., 2011). Table 5 summarizes recently reported fluorescent microbial biosensors in the literature.

Green fluorescent protein (*gfp*) has been widely used as a reporter gene and is fused to the host gene that allows reporter activity to be examined in individual cells. The *gfp* has some advantages such as stability, applicability for long term exposure, sensitivity to low concentrations, and inability of other microorganisms to produce it. However, it has some disadvantages such as the delay between protein production and fluorescence (Eltzov and Marks, 2011; Lei et al., 2006a; Su et al., 2011).

Kohlmeier et al. (2008) using an engineered *P. putida* whole-cell biosensor to determine bioavailable naphthalene, showed great consistency between their biosensor and the results from a Tenax extraction with chemical analysis while the detection speed was much faster. Fiorentino et al. (2009) developed a microbial biosensor using genetically engineered *E. coli* equipped with *gfp* for measuring aromatic aldehydes in aqueous systems. *GFP* was expressed under the control of an alcohol dehydrogenase-induced promoter. This biosensor showed a linear response in the millimolar range and was able to differentiate between various aromatic aldehydes. Stiner and Halverson (2002) developed a biosensor based on a *gfp* using the *P. fluorescens* strain. This biosensor was constructed and characterized for its potential to measure benzene, toluene, ethyl benzene, and related compounds in

Table 5
Fluorescent microbial biosensors.

Target	Microorganisms	Detection limit	References
Ni^{2+} and Co^{2+}	*Ralstonia eutropha* AE2515	0.0.06 Ni^{2+}, 0.5 Co^{2+} mg/L	Tibazarwa et al. (2001)
Urinary mercury (II)	*E. coli* MC1061	1.33×10^{-9} mg/L	Roda et al. (2001)
Hg^{2+}	*E. coli* HMS174	0.2 ng/g	Rasmussen et al. (2000)
Bioavailable copper	*P. fluorescens* DF57	0.3 mg/L	Tom-Petersen et al. (2001)
Bioavailable phosphorus	*Synechococcus* PCC 7942 reporter strain	0.01 mg/L	Schreiter et al. (2001)
Bioavailable naphthalene	*P. putida* carrying NAH7 plasmid	0.006–0.064 mg/L	Werlen et al. (2004)
Tributyltin	Bioluminescent recombinant *E. coli*	0.435 mg/L	Thouand et al. (2003)
Halogenated organic acids	Recombinant *E. coli*	>100 mg/L	Tauber et al. (2001)
Water pollutants/toxicity	*E. coli* HB101 pUCD607	0.09 to 21 mg/L	Horsburgh et al. (2002)
Methyl viologen	*E. coli* PGRFM	0.6–19.3 mg/L	Niazi et al. (2008)
3,4-Dichloropheno	*V. fischeri*	7–90 mg/L	Stolper et al. (2008)
2,4-Dichlorophenol	*V. fischeri*	40–200 mg/L	Stolper et al. (2008)
3,5-Dichlorophenol	*V. fischeri*	30–100 mg/L	Stolper et al. (2008)
2,3,5,6-Tetrachlorophenol	*V. fischeri*	4–30 mg/L	Stolper et al. (2008)
2,3,4,6-Tetrachlorophenol	*V. fischeri*	5–40 mg/L	Stolper et al. (2008)
Toluene (bioavailable BTEX)	*E. coli* DH5αpTOLLUX	0.7 mg/L	Li et al. (2008)
Chloramphenicol	*E. coli* EMS310	1 mg/L	Shapiro et al. (2005)
Hg^{2+}	*E. coli*	74 mg/L	Hakkila et al. (2004)
As^{2+}	*E. coli*	58 mg/L	Hakkila et al. (2004)
EDC	*Saccharomyces cerevisiae*	20 μg/L	Hollis et al. (2000)
Mitomycin C	*Photobacterium phosphoreum* (A2)	0.1 mg/L	Yaliang et al. (2004)
Naphthalene and salicylate	*P. fluorescens*HK44	1.2 and 0.5 mg/L	Trogl et al. (2005)
Cu	*Alcaligenes eutrophus*	0.063 mg/L	Leth et al. (2002)
Hydrogen peroxide	*E. coli* GC2	29.9 mg/L	Yoo et al. (2007)
Pollution- induced stress	*P. fluorescens* pUCD607	2 mg/L	Porteous et al. (2000)
Genotoxicant	*E. coli* DPD1718 containing recA'::lux fusion	0.1 mg/L mitomycin	Polyak et al. (2000)
ClO_2 gas	*P. fluorescens* 5RL	0.5 mg/L	del Busto-Ramos et al. (2008)

aqueous solutions. The *gfp* reporter gene microbial biosensor has been used to detect bioavailable toluene and related compounds (Casavant et al., 2003), N-acyl homoserine lactones in soil (Burmølle et al., 2003), water availability in microbial habitats (Axtell and Beattie, 2002), and for monitoring cell populations (Fesenko et al., 2005).

Similarly, in vitro fluorescent biosensors have been used for the assessment of environmental pollutants. Lin et al. (2006) developed a fluorescent BOD biosensor by immobilizing a mixed culture of microorganisms onto a dissolved oxygen optical fiber in an ormosil-PVA matrix. The developed biosensor was found to be more effective and showed high performance compared to the traditional BOD_5 method. In another study, Nguyen-Ngoc and Tran-Minh (2007) used chlorophyll from *C. vulgaris* as a fluorescent compound for anti- photosystem II (*PSII*) herbicide detection. Whole cells of *C. vulgaris* were immobilized in an inorganic translucent sol-gel matrix. Chlorophyll could be metabolized by *PSII*. The presence of anti-*PSII* herbicide (e.g. diuron) would inhibit the electron transfer in the *PSII* and result in an increase of chlorophyll fluorescence emission.

4.2.2. The bioluminescent biosensors

The bioluminescent sensing technique used in microbial biosensors is based on the change in luminescence emitted by living microorganisms in the presence of a target analyte (Lei et al., 2006a; Su et al., 2011). The *lux* gene, encoding luciferase, is the most popular reporter gene in bioluminescent microbial biosensors (Borisov and Wolfbeis, 2008). Luciferase catalyzes the oxidation of flavin mononucleotide (FMNH2) and the long-chain fatty aldehyde (RCHO) by oxygen with the emission of blue-green light (Hassan and Oh, 2010; Meighen, 1991). The bioluminescence provides a faster and more sensitive detection approach than fluorescence (Belkin, 2003). The expression of the *lux* gene can be controlled in either a constitutive or an inducible manner (Lei et al., 2006a; Su et al., 2011). In the constitutive manner, the *lux* gene is expressed continuously in the sensing microbe and bioluminescence will change directly with how the microbe reacts to the addition of the target analyte. This kind of reporter is good for evaluating the total toxicity of a contaminant.

A *V. fischeri*-based bioluminescent microbial biosensor has been developed for the rapid determination of toxic environmental pollutants in continuous flow systems. The light intensity produced by *V. fischeri* is reduced in the presence of toxic compounds (Stolper et al., 2008). Similarly, bioluminescent microbial biosensors based on the *lux* CDABE marked *Acinetobacter* sp. bacterium was used to assay the toxicity of a wastewater contaminated by heavy metals (Abd-El-Haleem et al., 2006). In the inducible manner, the *lux* gene is fused to a promoter regulated by the concentration of the compound of interest. As a result, the concentration of the compound can be quantitatively analyzed by detecting the bioluminescence intensity (Belkin, 2003; Rensing and Maier, 2003). Table 6 summarizes recently reported bioluminescent microbial biosensors in the literature.

Bioluminescent microbial biosensors have been used to monitor bioavailable heavy metals in the environment. Tibazarwa et al. (2001) used a whole cell biosensor containing *Ralstonia eutropha* AE2515 for the detection of bio-available heavy metals. The biosensor was constructed by transcriptionally fusing *cnr*YXH regulatory genes to the bioluminescent *lux*CDABE report system for the detection of bioavailable Ni^{2+} and Co^{2+} in soil. Similarly, bioavailable copper in soil is monitored by engineering *P. fluorescens* through the mutagenesis of the copper-induced gene and the *Tn5*::*lux*AB promoter probe transposon (Tom-Petersen et al., 2001). Bioluminescent microbial biosensors using the inducible reporter gene have also been developed for the measurement of bioavailable naphthalene (Werlen et al., 2004), tributyltin (Thouand et al., 2003), and halogenated organic acids (Tauber et al., 2001).

4.3. Colorimetric microbial biosensors

Colorimetric microbial biosensors based on the generation of colored compounds can be measured and correlated to the concentration of analytes (Su et al., 2011). A sensitive biosensor based on the color changes of living cells of fish in the presence of microbial toxins has been reported by Lei et al. (2006a). In the presence of toxins produced by microbial pathogens, the cells undergo visible color change and the color change depends on concentration of the toxin. The results suggest this cell-based biosensor's potential application in the detection and identification of virulence activity associated with certain air-, food-, and water-borne bacterial pathogens (Lei et al., 2006a).

A colorimetric whole cell biosensor for the detection of environmental pollutants such as benzene, toluene, ethyl benzene, and xylene (BTEX) was developed by Xu et al. (2003). The bioassay was based on the toluene dioxygenase-peroxidase enzyme which catalyzes the conversion of the BTEX components to their respective catechols followed by reaction with H_2O_2 in presence of horseradish peroxidase to colorimetric products that can be measured at 420_{nm}; the intensity of color was correlated to the concentration of BTEX.

Yoshida et al., 2008 developed a novel colorimetric biosensor based on the activity of the photosynthetic bacterium *Rhodopseudomonas palustris* no. 7. The biosensor changed color from green-yellow toward red (in a dose-dependent manner) in the presence of arsenite, and the change was obvious to the naked eye after 24 h without further manipulation. Also, this colorimetric biosensor could be used to monitor groundwater samples for the presence of arsenite in a variety of locations, even where electricity is not available.

5. Conclusions

In order to assess the toxic chemicals in water, industrial effluents, wastewater, and stream water, bioassays and biosensors have been developed. The development and application of toxicity assessment based on biological assays is increasing at a rapid rate. Many biological assays based on eukaryotes and prokaryotes are now available and

Table 6
Bioluminescent microbial biosensors.

Target	Microorganisms	Detection limit	References
Bioavailable iron	Recombinant *Pseudomonas syringae*	0.6–0.4 mg/L	Joyner and Lindow (2000)
Bioavailable toluene	*P. fluorescens* A506	0.02 mg/L	Casavant et al. (2003)
BOD	Sea water microorganisms	4 mg/l	Dai et al. (2004)
BOD	*P. putida* and optical fiber sensor	0.5 mg/l	Chee et al. (2000)
BOD	*B. licheniformis*	0.2 mg/L	Lin et al. (2006)
Lysine	*E. coli* lysA mini-Tn5-Km-gfpmut3	3 mg/L	Chalova et al. (2007)
Tetracycline	*E. coli* MC4100/pTGM	5 ng/mL–16 mg/L	Bahl et al. (2005)
Toluene	*P. putida* mt-2,	18.4 mg/L	Tizzard et al. (2006)
Toluene (bioavailable BTEX)	*E. coli* DH5 pTOLGFP	2.3 mg/L	Li et al. (2008)
Diuron (herbicide)	*C. vulgaris*	1 μg/L for	Nguyen-Ngoc and Tran-Minh (2007)
Uranium	*C. crescentus* NJH371	0.11 mg/L	Hillson et al. (2007)
Zinc	*B. megaterium* pSD202	0.07 mg/L	Date et al. (2007)
Mitomycin C	*E. coli* MG1655	2.5 ng/g soil	Norman et al. (2006)

commercialized. However, it is difficult to compare the sensitivities of these bioassays as there are many variations and differences in procedures. Furthermore, environmental conditions such as pH, temperature, and periods of incubation can all generate different responses from different bioassay tests. Most of toxicity assessments of recent studies have dealt with the use of the daphnids, bioluminescence bacteria (*Photobacterium phosphoreum* and *V. fischeri*), and the Microtox assay. Most environmental biosensor studies address sensitivity, selectively, portability, eco-friendliness, and electrical demand. Biosensors technologies for environmental application have continued to show advances in a wide range of areas. However, some of these biosensors cannot be used directly for toxicity assessment in stream water or wastewater without sample preparation which makes the process time consuming. Further research is required to address several drawbacks associated with some biosensors.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2014R1A1A2057095).

References

Abd-El-Haleem, D., Zaki, S., Abulhamd, A., Elbery, H., Abu-Elreesh, G., 2006. Acinetobacter bioreporter assessing heavy metals toxicity. J. Basic Microbiol. 46, 339–347.
Axtell, C.A., Beattie, G.A., 2002. Construction and characterization of a proU-*gfp* transcriptional fusion that measures water availability in a microbial habitat. Appl. Environ. Microbiol. 68, 4604–4612.
Bahl, M.I., Hansen, L.H., Sorensen, S.J., 2005. Construction of an extended range whole-cell tetracycline biosensor by use of the tet(M) resistance gene. FEMS Microbiol. Lett. 253, 201–205.
Barata, C., Baird, D.J., Nogueira, A.J.A., Soares, A.M.V.M., Riva, M.C., 2006. Toxicity of binary mixtures of metals and pyrethroid insecticides to *Daphnia magna* Straus. Implications for multi-substance risks assessment. Aquat. Toxicol. 78, 1–14.
Barata, C., Damasio, J., Lopez, M.A., Kuster, M., De Alda, M.L., Barceló, D., Riva, M.C., Raldua, D., 2007. Combined use of biomarkers and in situ bioassays in *Daphnia magna* to monitor environmental hazards of pesticides in the field. Environ. Toxicol. Chem. 26, 370–379.
Belkin, S., 2003. Microbial whole-cell sensing systems of environmental pollutants. Curr. Opin. Microbiol. 6, 206–212.
Bentley, A., Atkinson, A., Jezek, J., Rawson, D.M., 2001. Whole cell biosensors — electrochemical and optical approaches to ecotoxicity testing. Toxicol. in Vitro 15, 469–475.
Biran, I., Babai, R., Levcov, K., Rishpon, J., Ron, E.Z., 2000. Online and in situ monitoring of environmental pollutants: electrochemical biosensing of cadmium. Environ. Microbiol. 2, 285–290.
Bobacka, J., Ivaska, A., Lewenstam, A., 2008. Potentiometric ion sensors. Chem. Rev. 108, 329–351.
Borisov, S.M., Wolfbeis, O.S., 2008. Optical biosensors. Chem. Rev. 108, 423–461.
Bousse, L., 1996. Whole cell biosensors. Sensors Actuators B Chem. 34, 270–275.
Brogan, K.L., Walt, D.R., 2005. Optical fiber-based sensors: application to chemical biology. Curr. Opin. Chem. Biol. 9, 494–500.
Burmølle, M., Hansen, L.H., Oregaard, G., Sørensen, S.J., 2003. Presence of N-acyl homoserine lactones in soil detected by a whole-cell biosensor and flow cytometry. Microb. Ecol. 45, 226–236.
Casavant, N.C., Thompson, D., Beattie, G.A., Phillips, G.J., Halverson, L.J., 2003. Use of a site-specific recombination-based biosensor for detecting bioavailable toluene and related compounds on roots. Environ. Microbiol. 5, 238–249.
Chalova, V.I., Kim, W.K., Woodward, C.L., Ricke, S.C., 2007. Quantification of total and bioavailable lysine in feed protein sources by a whole-cell green fluorescent protein growth-based *Escherichia coli* biosensor. Appl. Microbiol. Biotechnol. 76, 91–99.
Chan, C., Lehmann, M., Tag, K., Lung, M., Gotthard, K., Riedel, K., Gruendig, B., Renneberg, R., 1999. Measurement of biodegradable substances using the salt-tolerant yeast *Arxula adeninivorans* for a microbial sensor immobilized with poly(carbamoyl) sulfonate (PCS) part I: construction and characterization of the microbial sensor. Biosens. Bioelectron. 14, 131–138.
Chan, C., Lehmann, M., Chan, K., Chan, P., Gruendig, B., Kunze, G., Renneberg, R., 2000. Designing an amperometric thick-film microbial BOD sensor. Biosens. Bioelectron. 15, 343–353.
Chang, I.S., Jang, J.K., Gil, G.C., Kim, M., Kim, H.J., Cho, B.W., Kim, B.H., 2004. Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor. Biosens. Bioelectron. 19, 607–613.
Chee, G.J., Nomura, Y., Karube, I., 1999. Biosensor for the estimation of low biochemical oxygen demand. Anal. Chim. Acta 379, 185–191.
Chee, G.J., Nomura, Y., Ikebukuro, K., Karube, I., 2000. Optical fiber biosensor for the determination of low biochemical oxygen demand. Biosens. Bioelectron. 15, 371–376.
Chee, G.J., Nomura, Y., Ikebukuro, K., Karube, I., 2005. Development of photocatalytic biosensor for the evaluation of biochemical oxygen demand. Biosens. Bioelectron. 21, 67–73.
Chouteau, C., Dzyadevych, S., Durrieu, C., Chovelon, J.M., 2005. A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples. Biosens. Bioelectron. 21, 273–281.
Collings, A.F., Caruso, F., 1997. Biosensors: recent advances. Rep. Prog. Phys. 60, 1397–1445.
Couture, P., Blaise, C., Cluis, D., Bastien, C., 1989. Zirconium toxicity assessment using bacteria, algae and fish assays. Water Air Soil Pollut. 47, 87–100.
Dai, Y.J., Lin, L., Li, P.W., Chen, X., Wang, X.R., Wong, K.Y., 2004. Comparison of BOD optical fiber biosensors based on different microorganisms immobilized in ormosil matrixes. Int. J. Environ. Anal. Chem. 84, 607–617.
Dalzell, D.J.B., Christofi, N., 2002. An ATP luminescence method for direct toxicity assessment of pollutants impacting on the activated sewage sludge process. Water Res. 36, 1493–1502.
Date, A., Pasini, P., Daunert, S., 2007. Construction of spores for portable bacterial whole-cell biosensing systems. Anal. Chem. 79, 9391–9397.
del Busto-Ramos, M., Budzik, M., Corvalan, C., Morgan, M., Turco, R., Nivens, D., Applegate, B., 2008. Development of an online biosensor for in situ monitoring of chlorine dioxide gas disinfection efficacy. Appl. Microbiol. Biotechnol. 78, 573–580.
Dennison, M.J., Turner, A.P.F., 1995. Biosensors for environmental monitoring. Biotechnol. Adv. 13, 1–12.
Ding, L., Du, D., Zhang, X., Ju, H., 2008. Trends in cell-based electrochemical biosensors. Curr. Med. Chem. 15, 3160–3170.
dos Santos, L.F., Defrenne, L., Krebs-Brown, A., 2002. Comparison of three microbial assay procedures for measuring toxicity of chemical compounds: ToxAlert®10, CellSense and Biolog MT2 microplates. Anal. Chim. Acta 456, 41–54.
D'Souza, S.F., 2001. Microbial biosensors. Biosens. Bioelectron. 16, 337–353.
Eltzov, E., Marks, R., 2011. Whole-cell aquatic biosensors. Anal. Bioanal. Chem. 400, 895–913.
Emelyanova, E.V., Reshetilov, A.N., 2002. *Rhodococcus erythropolis* as the receptor of cell-based sensor for 2,4-dinitrophenol detection: effect of 'co-oxidation'. Process Biochem. 37, 683–692.
Farré, M., Barceló, D., 2003. Toxicity testing of wastewater and sewage sludge by biosensors, bioassays and chemical analysis. TrAC Trends Anal. Chem. 22, 299–310.
Ferrari, B., Radetski, C.M., Veber, A.-M., Ferard, J.-F., 1999. Ecotoxicological assessment of solid wastes: a combined liquid- and solid-phase testing approach using a battery of bioassays and biomarkers. Environ. Toxicol. Chem. 18, 1195–1202.
Ferrini, A.M., Mannoni, V., Carpico, G., Pellegrini, G.E., 2008. Detection and identification of β-lactam residues in milk using a hybrid biosensor. J. Agric. Food Chem. 56, 784–788.
Fesenko, D.O., Nasedkina, T.V., Prokopenko, D.V., Mirzabekov, A.D., 2005. Biosensing and monitoring of cell populations using the hydrogel bacterial microchip. Biosens. Bioelectron. 20, 1860–1865.
Fiorentino, G., Ronca, R., Bartolucci, S., 2009. A novel E. coli biosensor for detecting aromatic aldehydes based on a responsive inducible archaeal promoter fused to the green fluorescent protein. Appl. Microbiol. Biotechnol. 82, 67–77.
Gaberlein, S., Spener, F., Zaborosch, C., 2000. Microbial and cytoplasmic membrane-based potentiometric biosensors for direct determination of organophosphorus insecticides. Appl. Microbiol. Biotechnol. 54, 652–658.
Galindo, E., Bautista, D., Garcia, J.L., Quintero, R., 1990. Microbial sensor for penicillins using a recombinant strain of *Escherichia coli*. Enzym. Microb. Technol. 12, 642–646.
Gurung, A., Hassan, S.H.A., Oh, S.E., 2011. Assessing acute toxicity of effluent from a textile industry and nearby river waters using sulfur-oxidizing bacteria in continuous mode. Environ. Technol. 32, 1597–1604.
Gurung, A., Oh, S.-E., Kim, K.D., Shin, B.-S., 2012. Semi-continuous detection of toxic hexavalent chromium using a sulfur-oxidizing bacteria biosensor. J. Environ. Manag. 106, 110–112.
Hakkila, K., Green, T., Leskinen, P., Ivask, A., Marks, R., Virta, M., 2004. Detection of bioavailable heavy metals in EILATox-Oregon samples using whole-cell luminescent bacterial sensors in suspension or immobilized onto fibre-optic tips. J. Appl. Toxicol. 24, 333–342.
Han, T.S., Sasaki, S., Yano, K., Ikebukuro, K., Kitayama, A., Nagamune, T., Karube, I., 2002. Flow injection microbial trichloroethylene sensor. Talanta 57, 271–276.
Hassan, S.H.A., Oh, S.E., 2010. Improved detection of toxic chemicals by *Photobacterium phosphoreum* using modified Boss medium. J. Photochem. Photobiol. B Biol. 101, 16–21.
Hassan, S.H.A., Van Ginkel, S.W., Kim, S.M., Yoon, S.H., Joo, J.H., Shin, B.S., Jeon, B.H., Bae, W., Oh, S.E., 2010. Isolation and characterization of *Acidithiobacillus caldus* from a sulfur-oxidizing bacterial biosensor and its role in detection of toxic chemicals. J. Microbiol. Methods 82, 151–155.
Hassan, S.H.A., Kim, Y.S., Oh, S.-E., 2012. Power generation from cellulose using mixed and pure cultures of cellulose-degrading bacteria in a microbial fuel cell. Enzym. Microb. Technol. 51, 269–273.
Hassan, S.H.A., Gad El-Rab, S.M.F., Rahimnejad, M., Ghasemi, M., Joo, J.-H., Sik-Ok, Y., Kim, I.S., Oh, S.-E., 2014. Electricity generation from rice straw using a microbial fuel cell. Int. J. Hydrog. Energy 39, 9490–9496.
Hillson, N.J., Hu, P., Andersen, G.L., Shapiro, L., 2007. *Caulobacter crescentus* as a whole-cell uranium biosensor. Appl. Environ. Microbiol. 73, 7615–7621.
Hock, B., 1997. Antibodies for immunosensors. A review. Anal. Chim. Acta 347, 177–186.
Hollis, R.P., Killham, K., Glover, L.A., 2000. Design and application of a biosensor for monitoring toxicity of compounds to eukaryotes. Appl. Environ. Microbiol. 66, 1676–1679.
Homola, J., Yee, S.S., Gauglitz, G., 1999. Surface plasmon resonance sensors: review. Sensors Actuators B Chem. 54, 3–15.
Horsburgh, A.M., Mardlin, D.P., Turner, N.L., Henkler, R., Strachan, N., Glover, L.A., Paton, G.I., Killham, K., 2002. On-line microbial biosensing and fingerprinting of water pollutants. Biosens. Bioelectron. 17, 495–501.
Ibraheem, A., Campbell, R.E., 2010. Designs and applications of fluorescent protein-based biosensors. Curr. Opin. Chem. Biol. 14, 30–36.

Ikebukuro, K., Honda, M., Nakanishi, K., Nomura, Y., Masuda, Y., Yokoyama, K., Yamauchi, Y., Karube, I., 1996a. Flow-type cyanide sensor using an immobilized microorganism. Electroanalysis 8, 876–879.

Ikebukuro, K., Miyata, A., Cho, S.J., Nomura, Y., Chang, S.M., Yamauchi, Y., Hasebe, Y., Uchiyama, S., Karube, I., 1996b. Microbial cyanide sensor for monitoring river water. J. Biotechnol. 48, 73–80.

ISO, 2007. Water quality-test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation. International Organization for Standardization. W ISO 8192 (Geneva, Switzerland).

Jaffrezic-Renault, N., Dzyadevych, S.V., 2008. Conductometric microbiosensors for environmental monitoring. Sensors 8, 2569–2588.

Janssen, C.R., Persoone, G., Snell, T.W., 1994. Cyst-based toxicity tests. VIII. Short-chronic toxicity tests with the freshwater rotifer *Brachionus calyciflorus*. Aquat. Toxicol. 28, 243–258.

Jantra, J., Zilouei, H., Liu, J., Guieysse, B., Thavarungkul, P., Kanatharana, P., Mattiasson, B., 2005. Microbial biosensor for the analysis of 2,4 dichlorophenol. Anal. Lett. 38, 1071–1083.

Jia, J., Tang, M., Chen, X., Qi, L., Dong, S., 2003. Co-immobilized microbial biosensor for BOD estimation based on sol-gel derived composite material. Biosens. Bioelectron. 18, 1023–1029.

Joyner, D.C., Lindow, S.E., 2000. Heterogeneity of iron bioavailability on plants assessed with a whole-cell GFP-based bacterial biosensor. Microbiology 146, 2435–2445.

Karube, I., Matsunaga, T., Mitsuda, S., Suzuki, S., 1977. Microbial electrode BOD sensors. Biotechnol. Bioeng. 19, 1535–1547.

Karube, I., Yokoyama, K., Sode, K., Tamiya, E., 1989. Microbial bod sensor utilizing thermophilic bacteria. Anal. Lett. 22, 791–801.

Kim, M.N., Kwon, H.S., 1999. Biochemical oxygen demand sensor using *Serratia marcescens* LSY 4. Biosens. Bioelectron. 14, 1–7.

Kirgoz, U.A., Odaci, D., Timur, S., Merkoci, A., Pazarlioglu, N., Telefoncu, A., Alegret, S., 2006. Graphite epoxy composite electrodes modified with bacterial cells. Bioelectrochemistry 69, 128–131.

Kohlmeier, S., Mancuso, M., Deepthike, U., Tecon, R., van der Meer, J.R., Harms, H., Wells, M., 2008. Comparison of naphthalene bioavailability determined by whole-cell biosensing and availability determined by extraction with Tenax. Environ. Pollut. 156, 803–808.

König, A., Reul, T., Harmeling, C., Spener, F., Knoll, M., Zaborosch, C., 2000. Multimicrobial sensor using microstructured three-dimensional electrodes based on silicon technology. Anal. Chem. 72, 2022–2028.

Kulys, J., Kadziauskiene, K., 1980. Yeast BOD sensor. Biotechnol. Bioeng. 22, 221–226.

Kumar, S., Kundu, S., Pakshirajan, K., Dasu, V.V., 2008. Cephalosporins determination with a novel microbial biosensor based on permeabilized *Pseudomonas aeruginosa* whole cells. Appl. Biochem. Biotechnol. 151, 653–664.

Lawrence, A.J., Moores, G.R., 1972. Conductimetry in enzyme studies. Eur. J. Biochem. 24, 538–546.

Lee, J.I., Karube, I., 1996. Reactor type sensor for cyanide using an immobilized microorganism. Electroanalysis 8, 1117–1120.

Lehmann, M., Riedel, K., Adler, K., Kunze, G., 2000. Amperometric measurement of copper ions with a deputy substrate using a novel *Saccharomyces cerevisiae* sensor. Biosens. Bioelectron. 15, 211–219.

Lei, Y., Mulchandani, P., Chen, W., Wang, J., Mulchandani, A., 2003. A microbial biosensor for p-nitrophenol using *Arthrobacter* Sp. Electroanalysis. 15 (14), 1160–1164.

Lei, Y., Mulchandani, P., Chen, W., Wang, J., Mulchandani, A., 2004. *Arthrobacter sp.* JS443-based whole cell amperometric biosensor for *p*-nitrophenol. Electroanalysis 16, 2030–2034.

Lei, Y., Mulchandani, P., Chen, W., Mulchandani, A., 2005. Direct determination of *p*-nitrophenyl substituent organophosphorus nerve agents using a recombinant *Pseudomonas putida* js444-modified clark oxygen electrode. J. Agric. Food Chem. 53, 524–527.

Lei, Y., Chen, W., Mulchandani, A., 2006a. Microbial biosensors. Anal. Chim. Acta 568, 200–210.

Lei, Y., Mulchandani, P., Chen, W., Mulchandani, A., 2006b. Biosensor for direct determination of fenitrothion and EPN using recombinant *Pseudomonas putida* js444 with surface expressed organophosphorus hydrolase. 1. Modified clark oxygen electrode. Sensors 6, 466–472.

Leth, S., Maltoni, S., Simkus, R., Mattiasson, B., Corbisier, P., Klimant, I., Wolfbeis, O.S., Csöregi, E., 2002. Engineered bacteria based biosensors for monitoring bioavailable heavy metals. Electroanalysis 14, 35–42.

Li, Y.R., Chu, J., 1991. Study of BOD microbial sensors for waste water treatment control. Appl. Biochem. Biotechnol. 28-29, 855–863.

Li, Y.F., Li, F.Y., Ho, C.L., Liao, V.H.C., 2008. Construction and comparison of fluorescence and bioluminescence bacterial biosensors for the detection of bioavailable toluene and related compounds. Environ. Pollut. 152, 123–129.

Liao, J.-D., Wang, S.-H., Hsu, D.-J., 2001. Studies on the early detection of wastewater's toxicity using a microbial sensing system. Sensors Actuators B Chem. 72, 167–173.

Lin, L., Xiao, L.L., Huang, S., Zhao, L., Cui, J.S., Wang, X.H., Chen, X., 2006. Novel BOD optical fiber biosensor based on co-immobilized microorganisms in ormosils matrix. Biosens. Bioelectron. 21, 1703–1709.

Liu, J., Mattiasson, B., 2002. Microbial BOD sensors for wastewater analysis. Water Res. 36, 3786–3802.

Liu, J., Björnsson, L., Mattiasson, B., 2000. Immobilised activated sludge based biosensor for biochemical oxygen demand measurement. Biosens. Bioelectron. 14, 883–893.

Liu, M., Chen, C.M., Cheng, H., Chen, H., Su, Y., Hung, T., 2002. Toxicity of different industrial effluents in Taiwan: a comparison of the sensitivity of *Daphnia similis* and microtox®. Environ. Toxicol. 17, 93–97.

Logan, B.E., 2009. Exoelectrogenic bacteria that power microbial fuel cells. Nat. Rev. Microbiol. 7, 375–381.

Mak, K.K.W., Law, A.W.C., Tokuda, S., Yanase, H., Renneberg, R., 2005. Application of cyanide hydrolase from *Klebsiella* sp. in a biosensor system for the detection of low-level cyanide. Appl. Microbiol. Biotechnol. 67, 631–636.

Maltby, L., 2007. Small scale freshwater toxicity investigations: volume 1–toxicity test methods. Freshw. Biol. 52, 198-198.

Marazuela, M.D., Moreno-Bondi, M.C., 2002. Fiber-optic biosensors - an overview. Anal. Bioanal. Chem. 372, 664–682.

Marty, J.L., Olive, D., Asano, Y., 1997. Measurement of BOD: correlation between 5-day BOD and commercial BOD biosensor values. Environ. Technol. 18, 333–337.

Meighen, E.A., 1991. Molecular biology of bacterial bioluminescence. Microbiol. Rev. 55, 123–142.

Mikkelsen, S.R., Rechnitz, G.A., 1989. Conductometric transducers for enzyme-based biosensors. Anal. Chem. 61, 1737–1742.

Moulin, A.M., O'Shea, S.J., Welland, M.E., 2000. Microcantilever-based biosensors. Ultramicroscopy 82, 23–31.

Mulchandani, A., Mulchandani, P., Kaneva, I., Chen, W., 1998a. Biosensor for direct determination of organophosphate nerve agents using recombinant *Escherichia coli* with surface-expressed organophosphorus hydrolase. 1. Potentiometric microbial electrode. Anal. Chem. 70, 4140–4145.

Mulchandani, A., Mulchandani, P., Chauhan, S., Kaneva, I., Chen, W., 1998b. A potentiometric microbial biosensor for direct determination of organophosphate nerve agents. Electroanalysis 10, 733–737.

Mulchandani, P., Lei, Y., Chen, W., Wang, J., Mulchandani, A., 2002. Microbial biosensor for p-nitrophenol using *Moraxella* sp. Anal. Chim. Acta 470, 79–86.

Mulchandani, P., Hangarter, C.M., Lei, Y., Chen, W., Mulchandani, A., 2005. Amperometric microbial biosensor for *p*-nitrophenol using *Moraxella sp.*-modified carbon paste electrode. Biosens. Bioelectron. 21, 523–527.

Mulchandani, P., Chen, W., Mulchandani, A., 2006. Microbial biosensor for direct determination of nitrophenyl-substituted organophosphate nerve agents using genetically engineered *Moraxella* sp. Anal. Chim. Acta 568, 217–221.

Munkittrick, K.R., Power, E.A., Sergy, G.A., 1991. The relative sensitivity of microtox, daphnid, rainbow trout, and fathead minnow acute lethality tests. Environ. Toxicol. Water Qual. 6, 35–62.

Nakanishi, K., Ikebukuro, K., Karube, I., 1996. Determination of cyanide using a microbial sensor. Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology. 60, 97–106.

Neufeld, T., Biran, D., Popovtzer, R., Erez, T., Ron, E.Z., Rishpon, J., 2006. Genetically engineered pfabA pfabR bacteria: an electrochemical whole cell biosensor for detection of water toxicity. Anal. Chem. 78, 4952–4956.

Nguyen-Ngoc, H., Tran-Minh, C., 2007. Fluorescent biosensor using whole cells in an inorganic translucent matrix. Anal. Chim. Acta 583, 161–165.

Niazi, J.H., Kim, B.C., Ahn, J.M., Gu, M.B., 2008. A novel bioluminescent bacterial biosensor using the highly specific oxidative stress-inducible pgi gene. Biosens. Bioelectron. 24, 670–675.

Norman, A., Hansen, L.H., Sorensen, S.J., 2006. A flow cytometry-optimized assay using an SOS-green fluorescent protein (SOS-GFP) whole-cell biosensor for the detection of genotoxins in complex environments. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 603, 164–172.

Odaci, D., Sezginturk, M.K., Timur, S., Pazarlioglu, N., Pilloton, R., Dinckaya, E., Telefoncu, A., 2009. *Pseudomonas putida* based amperometric biosensors for 2,4-D detection. Prep. Biochem. Biotechnol. 39, 11–19.

OECD, 2014. OECD Series on Testing and Assessment Guidance on Grouping of Chemicals. OECD Publishing.

Oh, S.E., Hassan, S.H.A., Van Ginkel, S.W., 2011. A novel biosensor for detecting toxicity in water using sulfur-oxidizing bacteria. Sensors Actuators B Chem. 154, 17–21.

Ohki, A., Shinohara, K., Ito, O., Naka, K., Maeda, S., Sato, T., Akano, H., Kato, N., Kawamura, Y., 1994. A BOD sensor using *Klebsiella oxytoca* AS1. Int. J. Environ. Anal. Chem. 56, 261–269.

Okochi, M., Mima, K., Miyata, M., Shinozaki, Y., Haraguchi, S., Fujisawa, M., Kaneko, M., Masukata, T., Matsunaga, T., 2004. Development of an automated water toxicity biosensor using *Thiobacillus ferrooxidans* for monitoring cyanides in natural water for a water filtering plant. Biotechnol. Bioeng. 87, 905–911.

Ou, Z., Sun, T., Zhang, H., 1994. A bioassay for determining simazine in water using aquatic flowering plants (*Ceratophyllum oryzetorum*, *Ranunculus trichophyllus* and *Alisma plantago-aquatica*). Pestic. Sci. 42, 173–178.

Polak, M.E., Rawson, D.M., Haggett, B.G.D., 1996. Redox mediated biosensors incorporating cultured fish cells for toxicity assessment. Biosens. Bioelectron. 11, 1253–1257.

Polyak, B., Bassis, E., Novodvorets, A., Belkin, S., Marks, R.S., 2000. Optical fiber bioluminescent whole-cell microbial biosensors to genotoxicants. Water Sci. Technol. 42, 305–311.

Porteous, F., Killham, K., Meharg, A., 2000. Use of a lux-marked *Rhizobacterium* as a biosensor to assess changes in rhizosphere C flow due to pollutant stress. Chemosphere 41, 1549–1554.

Pretti, C., Chiappe, C., Baldetti, I., Brunini, S., Monni, G., Intorre, L., 2009. Acute toxicity of ionic liquids for three freshwater organisms: *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*. Ecotoxicol. Environ. Saf. 72, 1170–1176.

Price, E.E., Donahue, M.J., Dickson, K.L., Rodgers Jr., J.H., 1990. Effects of elevated calcium concentration on Na-K-ATPase activity of two euryhaline species *Cyprinodon variegatus* and *Mysidopsis bahia*. Bull. Environ. Contam. Toxicol. 44, 121–128.

Rainina, E.I., Efremenco, E.N., Varfolomeyev, S.D., Simonian, A.L., Wild, J.R., 1996. The development of a new biosensor based on recombinant *E. coli* for the direct detection of organophosphorus neurotoxins. Biosens. Bioelectron. 11, 991–1000.

Rajasekar, S., Rajasekar, R., Narasimhan, K.C., 2000. *Acetobacter peroxydans* based electrochemical biosensor for hydrogen peroxide. Bull. Electrochem. 16, 25–28.

Ramanathan, K., Danielsson, B., 2001. Principles and applications of thermal biosensors. Biosens. Bioelectron. 16, 417–423.

Rasinger, J.D., Marrazza, G., Briganti, F., Scozzafava, A., Mascini, M., Turner, A.P.F., 2005. Evaluation of an FIA operated amperometric bacterial biosensor, based on *Pseudomonas putida* F1 for the detection of benzene, toluene, ethylbenzene, and xylenes (BTEX). Anal. Lett. 38, 1531–1547.

Rasmussen, L.D., Sorensen, S.J., Turner, R.R., Barkay, T., 2000. Application of a mer-lux biosensor for estimating bioavailable mercury in soil. Soil Biol. Biochem. 32, 639–646.

Rastogi, S., Kumar, A., Mehra, N.K., Makhijani, S.D., Manoharan, A., Gangal, V., Kumar, R., 2003. Development and characterization of a novel immobilized microbial membrane for rapid determination of biochemical oxygen demand load in industrial waste-waters. Biosens. Bioelectron. 18, 23–29.

Rensing, C., Maier, R.M., 2003. Issues underlying use of biosensors to measure metal bioavailability. Ecotoxicol. Environ. Saf. 56, 140–147.

Riedel, K., Renneberg, R., Kühn, M., Scheller, F., 1988. A fast estimation of biochemical oxygen demand using microbial sensors. Appl. Microbiol. Biotechnol. 28, 316–318.

Riedel, K., Lehmann, M., Tag, K., Renneberg, R., Kunze, G., 1998. *Arxula adeninivorans* based sensor for the estimation of BOD. Anal. Lett. 31, 1–12.

Roda, A., Pasini, P., Mirasoli, M., Guardigli, M., Russo, C., Musiani, M., Baraldini, M., 2001. Sensitive determination of urinary mercury(II) by a bioluminescent transgenic bacteria-based biosensor. Anal. Lett. 34, 29–41.

Rodriguez-Mozaz, S., Marco, M.-P., Lopez de Alda, Maria J., Barceló, D., 2004. Biosensors for environmental monitoring of endocrine disruptors: a review article. Anal. Bioanal. Chem. 378, 588–598.

Rodriguez-Mozaz, S., Alda, M.J.L.D., Marco, M.-P., Barceló, D., 2005. Biosensors for environmental monitoring: A global perspective. Talanta 65, 291–297.

Rosenfeldt, E.J., Chen, P.J., Kullman, S., Linden, K.G., 2007. Destruction of estrogenic activity in water using UV advanced oxidation. Sci. Total Environ. 377, 105–113.

Rotariu, L., Bala, C., Magearu, V., 2004. New potentiometric microbial biosensor for ethanol determination in alcoholic beverages. Anal. Chim. Acta 513, 119–123.

Ruebhart, D.R., Cock, I.E., Shaw, G.R., 2008. Brine shrimp bioassay: importance of correct taxonomic identification of *Artemia* (*Anostraca*) species. Environ. Toxicol. 23, 555–560.

Sangeetha, S., Sugandhi, G., Murugesan, M., Murali Madhav, V., Berchmans, S., Rajasekar, R., Rajasekar, S., Jeyakumar, D., Prabhakara Rao, G., 1996. Torulopsis *Candida* Based Sensor for the estimation of biochemical oxygen demand and its evaluation. Electroanalysis 8, 698–701.

Sarakinos, H.C., Bermingham, N., White, P.A., Rasmussen, J.B., 2000. Correspondence between whole effluent toxicity and the presence of priority substances in complex industrial effluents. Environ. Toxicol. Chem. 19, 63–71.

Schreiter, P.P.Y., Gillor, O., Post, A., Belkin, S., Schmid, R.D., Bachmann, T.T., 2001. Monitoring of phosphorus bioavailability in water by an immobilized luminescent cyanobacterial reporter strain. Biosens. Bioelectron. 16, 811–818.

Shapiro, E., Lu, C., Baneyx, F., 2005. A set of multicolored *Photinus pyralis* luciferase mutants for *in vivo* bioluminescence applications. Protein Eng. Des. Sel. 18, 581–587.

Shijin, R., 2004. Assessing wastewater toxicity to activated sludge: recent research and developments. Environ. Int. 30, 1151–1164.

Shitanda, I., Takada, K., Sakai, Y., Tatsuma, T., 2005. Compact amperometric algal biosensors for the evaluation of water toxicity. Anal. Chim. Acta 530, 191–197.

Shitanda, I., Takamatsu, S., Watanabe, K., Itagaki, M., 2009. Amperometric screen-printed algal biosensor with flow injection analysis system for detection of environmental toxic compounds. Electrochim. Acta 54, 4933–4936.

Shul'ga, A.A., Soldatkin, A.P., El'skaya, A.V., Dzyadevich, S.V., Patskovsky, S.V., Strikha, V.I., 1994. Thin-film conductometric biosensors far glucose and urea determination. Biosens. Bioelectron. 9, 217–223.

Snell, T.W., Moffat, B.D., Janssen, C., Persoone, G., 1991. Acute toxicity tests using rotifers. III. Effects of temperature, strain, and exposure time on the sensitivity of *Brachionus plicatilis*. Environ. Toxicol. Water Qual. 6, 63–75.

Stein, N.E., Hamelers, H.V.M., Buisman, C.N.J., 2010. Stabilizing the baseline current of a microbial fuel cell-based biosensor through overpotential control under non-toxic conditions. Bioelectrochemistry 78, 87–91.

Stiner, L., Halverson, L.J., 2002. Development and characterization of a green fluorescent protein-based bacterial biosensor for bioavailable toluene and related compounds. Appl. Environ. Microbiol. 68, 1962–1971.

Stolper, P., Fabel, S., Weller, M.G., Knopp, D., Niessner, R., 2008. Whole-cell luminescence-based flow-through biodetector for toxicity testing. Anal. Bioanal. Chem. 390, 1181–1187.

Su, L., Jia, W., Hou, C., Lei, Y., 2011. Microbial biosensors: a review. Biosens. Bioelectron. 26, 1788–1799.

Suter Ii, G.W., Rosen, A.E., 1988. Comparative toxicology for risk assessment of marine fishes and crustaceans. Environ. Sci. Technol. 22, 548–556.

Svenson, A., Sandén, B., Dalhammar, G., Remberger, M., Kaj, L., 2000. Toxicity identification and evaluation of nitrification inhibitors in wastewaters. Environ. Toxicol. 15, 527–532.

Tag, K., Kwong, A.W.K., Lehmann, M., Chan, C., Renneberg, R., Riedel, K., Kunze, G., 2000. Fast detection of high molecular weight substances in wastewater based on an enzymatic hydrolysis combined with the Arxula BOD sensor system. J. Chem. Technol. Biotechnol. 75, 1080–1082.

Tag, K., Riedel, K., Bauer, H.J., Hanke, G., Baronian, K.H.R., Kunze, G., 2007. Amperometric detection of Cu^{2+} by yeast biosensors using flow injection analysis (FIA). Sensors Actuators B Chem. 122, 403–409.

Tan, T.C., Qian, Z., 1997. Dead *Bacillus subtilis* cells for sensing biochemical oxygen demand of waters and wastewaters. Sensors Actuators B Chem. 40, 65–70.

Tan, T.C., Li, F., Neoh, K.G., Lee, Y.K., 1992. Microbial membrane-modified dissolved oxygen probe for rapid biochemical oxygen demand measurement. Sensors Actuators B Chem. 8, 167–172.

Tan, T.C., Li, F., Neoh, K.G., 1993. Measurement of BOD by initial rate of response of a microbial sensor. Sensors Actuators B Chem. 10, 137–142.

Taranova, L., Semenchuk, I., Manolov, T., Iliasov, P., Reshetilov, A., 2002. Bacteria-degraders as the base of an amperometric biosensor for detection of anionic surfactants. Biosens. Bioelectron. 17, 635–640.

Tauber, M., Rosen, R., Belkin, S., 2001. Whole-cell biodetection of halogenated organic acids. Talanta 55, 959–964.

Thévenot, D.R., Toth, K., Durst, R.A., Wilson, G.S., 2001. Electrochemical biosensors: recommended definitions and classification. Biosens. Bioelectron. 16, 121–131.

Thouand, G., Horry, H., Durand, M.J., Picart, P., Bendriaa, L., Daniel, P., DuBow, M.S., 2003. Development of a biosensor for on-line detection of tributyltin with a recombinant bioluminescent *Escherichia coli* strain. Appl. Microbiol. Biotechnol. 62, 218–225.

Tibazarwa, C., Corbisier, P., Mench, M., Bossus, A., Solda, P., Mergeay, M., Wyns, L., Van der Lelie, D., 2001. A microbial biosensor to predict bioavailable nickel in soil and its transfer to plants. Environ. Pollut. 113, 19–26.

Timur, S., Pazarlioğlu, N., Pilloton, R., Telefoncu, A., 2003. Detection of phenolic compounds by thick film sensors based on *Pseudomonas putida*. Talanta 61, 87–93.

Timur, S., Seta, L.D., Pazarlioğlu, N., Pilloton, R., Telefoncu, A., 2004. Screen printed graphite biosensors based on bacterial cells. Process Biochem. 39, 1325–1329.

Timur, S., Anik, U., Odaci, D., Gorton, L., 2007a. Development of a microbial biosensor based on carbon nanotube (CNT) modified electrodes. Electrochem. Commun. 9, 1810–1815.

Timur, S., Haghighi, B., Tkac, J., Pazarlioglu, N., Telefoncu, A., Gorton, L., 2007b. Electrical wiring of *Pseudomonas putida* and *Pseudomonas fluorescens* with osmium redox polymers. Bioelectrochemistry 71, 38–45.

Tišler, T., Zagorc-Končan, J., Cotman, M., Drolc, A., 2004. Toxicity potential of disinfection agent in tannery wastewater. Water Res. 38, 3503–3510.

Tizzard, A.C., Bergsma, J.H., Lloyd-Jones, G., 2006. A resazurin-based biosensor for organic pollutants. Biosens. Bioelectron. 22, 759–763.

Tom-Petersen, A., Hosbond, C., Nybroe, O., 2001. Identification of copper-induced genes in *Pseudomonas fluorescens* and use of a reporter strain to monitor bioavailable copper in soil. FEMS Microbiol. Ecol. 38, 59–67.

Tothill, I., Turner, A., 1996a. Developments in bioassay methods for toxicity testing in water treatment. TrAC Trends Anal. Chem. 15, 178–188.

Tothill, I.E., Turner, A.P.F., 1996b. Developments in bioassay methods for toxicity testing in water treatment. TrAC Trends Anal. Chem. 15, 178–188.

Trogl, J., Ripp, S., Kuncova, G., Sayler, G.S., Churava, A., Parik, P., Demnerova, K., Halova, J., Kubicova, L., 2005. Selectivity of whole cell optical biosensor with immobilized bioreporterPseudomonas fluorescens HK44. Sensors Actuators B Chem. 107, 98–103.

Tront, J.M., Fortner, J.D., Plötze, M., Hughes, J.B., Puzrin, A.M., 2008. Microbial fuel cell biosensor for *in situ* assessment of microbial activity. Biosens. Bioelectron. 24, 586–590.

USEPA, 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters toFreshwater and Marine Organisms, fifth ed. U.S. Environmental Protection Agency.in: Office of Water (4303T), ed. 1200 Pennsylvania Avenue, NW, Washington, DC20460. EPA-821-R-02-012.

Van Ginkel, S.W., Hassan, S.H.A., Oh, S.E., 2010. Detecting endocrine disrupting compounds in water using sulfur-oxidizing bacteria. Chemosphere 81, 294–297.

Van Ginkel, S.W., Hassan, S.H.A., Ok, Y.S., Yang, J.E., Kim, Y.S., Oh, S.E., 2011. Detecting oxidized contaminants in water using sulfur-oxidizing bacteria. Environ. Sci. Technol. 45, 3739–3745.

Verma, N., Singh, M., 2003. A disposable microbial based biosensor for quality control in milk. Biosens. Bioelectron. 18, 1219–1224.

Wang, J., 2002. Electrochemical nucleic acid biosensors. Anal. Chim. Acta 469, 63–71.

Wang, J., 2008. Electrochemical glucose biosensors. Chem. Rev. 108, 814–825.

Werlen, C., Jaspers, M.C.M., Van Der Meer, J.R., 2004. Measurement of biologically available naphthalene in gas and aqueous phases by use of a *Pseudomonas putida* biosensor. Appl. Environ. Microbiol. 70, 43–51.

Xu, Z., Mulchandani, A., Chen, W., 2003. Detection of benzene, toluene, ethyl benzene, and xylenes (BTEX) using toluene dioxygenase-peroxidase coupling reactions. Biotechnol. Prog. 19, 1812–1815.

Yaliang, S., Ticho, Z., Jianli, G., Yiyong, L., 2004. Dark variants of luminous bacteria whole cell bioluminescent optical fiber sensor to genotoxicants. Journal of Huazhong University of Science and Technology – Medical Sciences 24, 507–509.

Yang, Z., Sasaki, S., Karube, I., Suzuki, H., 1997. Fabrication of oxygen electrode arrays and their incorporation into sensors for measuring biochemical oxygen demand. Anal. Chim. Acta 357, 41–49.

Yoo, S.K., Lee, J.H., Yun, S.-S., Gu, M.B., Lee, J.H., 2007. Fabrication of a bio-MEMS based cell-chip for toxicity monitoring. Biosens. Bioelectron. 22, 1586–1592.

Yoshida, N., Yano, K., Morita, T., McNiven, S.J., Nakamura, H., Karube, I., 2000. A mediator-type biosensor as a new approach to biochemical oxygen demand estimation. Analyst 125, 2280–2284.

Yoshida, N., Hoashi, J., Morita, T., McNiven, S.J., Nakamura, H., Karube, I., 2001. Improvement of a mediator-type biochemical oxygen demand sensor for on-site measurement. J. Biotechnol. 88, 269–275.

Yoshida, K., Inoue, K., Takahashi, Y., Ueda, S., Isoda, K., Yagi, K., Maeda, I., 2008. Novel carotenoid-based biosensor for simple visual detection of arsenite: characterization and preliminary evaluation for environmental application. Appl. Environ. Microbiol. 74, 6730–6738.

Zhai, J., Cui, H., Yang, R., 1997. DNA based biosensors. Biotechnol. Adv. 15, 43–58.

Ziglio, G., Flaim, G., Siligardi, M., 2008. Biological Monitoring of Rivers. Wiley.

Zlatev, R., Magnin, J.P., Ozil, P., Stoytcheva, M., 2006a. Bacterial sensors based on *Acidithiobacillus* ferrooxidans: part I. Fe $^{2+}$ and $S_2O_3^{2-}$ determination. Biosens. Bioelectron. 21, 1493–1500.

Zlatev, R., Magnin, J.P., Ozil, P., Stoytcheva, M., 2006b. Bacterial sensors based on *Acidithiobacillus ferrooxidans*: part II. Cr(VI) determination. Biosens. Bioelectron. 21, 1501–1506.