Recent biosensing developments in environmental security

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Environmental security is one of the fundamental requirements of our well being. However, it still remains a major global challenge. Therefore, in addition to reducing and/or eliminating the amounts of toxic discharges into the environment, there is need to develop techniques that can detect and monitor these environmental pollutants in a sensitive and selective manner to enable effective remediation. Because of their integrated nature, biosensors are ideal for environmental monitoring and detection as they can be portable and provide selective and sensitive rapid responses in real time. In this review we discuss the main concepts behind the development of biosensors that have most relevant applications in the field of environmental monitoring and detection. We also review and document recent trends and challenges in biosensor research and development particularly in the detection of species of environmental significance such as organophosphate nerve agents, heavy metals, organic contaminants, pathogenic microorganisms and their toxins. Special focus will be given to the trends that have the most promising applications in environmental security. We conclude by highlighting the directions towards which future biosensors research in environmental security sector might proceed.

Introduction

The detection and monitoring of environmental pollutants in soil, water and air is very important in the overall safety and security of humans, other animals and plants. While highly sensitive and selective, traditional chromatography and spectroscopy analytical techniques are time consuming, expensive and require a lot of expertise. Therefore, there is need for simple, rapid, specific, sensitive and portable methods for analyzing environmental security threats.

A biosensor is an integrated device that consists of a biological recognition species in direct contact with a transduction element. Therefore, biosensors can be categorized according to the biological recognition element (immuno, enzymatic, DNA and whole-cell biosensors) or the signal transduction method (optical, mass-based, electrochemical, and thermal biosensors (Fig. 1)). Whatever the category, a biosensor simply combines a biological recognition element with a suitable transduction

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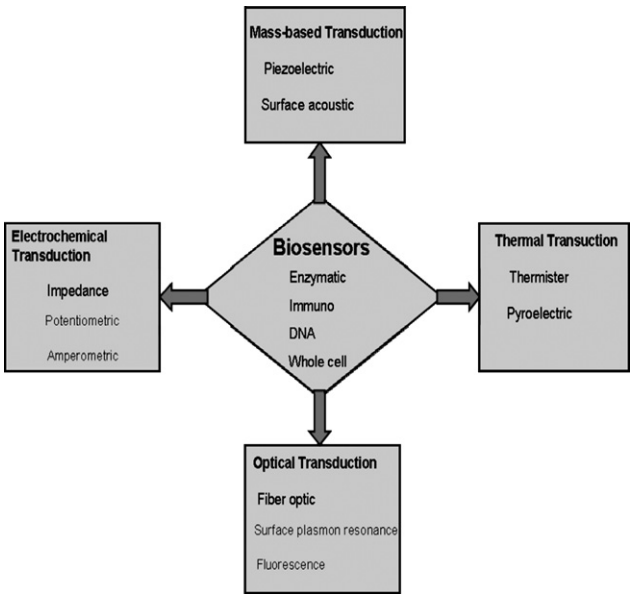

Fig. 1 Classification of biosensors.

method such that a meaningful signal can be realized when binding or some reaction occurs between that element with a target species. Because of their integrated nature, biosensors are ideal for environmental monitoring and detection as they can be portable and can provide rapid responses in real time. Additionally, the presence of the biological element within the biosensor system ensure extremely specific and highly sensitive responses. These factors make biosensors very attractive compared to contemporary chromatographic, spectroscopic techniques and bioassays in environmental sensing and detection.

Enzymes were among the first recognition elements to be incorporated into biosensors.[1] By acting as biocatalytic elements, enzymes enable the detection of analytes in various ways. Since enzymatic reaction are accompanied by the consumption or production of species such as CO_2, NH_3, H_2O_2, H^+ or O_2 various transducers easily detect and correlate this species to the substrates. Another way that enzymes can assist in detection is when substrates activate[2–4] or inhibit[5,6] enzyme or protein activity. The inhibition or activation is correlated to the substrate concentration. Some metals ions are good candidates for enzyme activation as those ions are required by enzyme sites in order to become active catalytically. On the other hand, enzyme inhibitors can also be measured by how much they decrease enzyme activity. A major advantage of enzyme-based biosensors is the ability, in some cases, to modify catalytic properties or substrate specificity by genetic engineering. The major limitation is the lack of specificity in differentiating among compounds of similar classes.

Immunosensors are inherently more versatile than enzyme-based biosensors because antibodies are more selective and specific. Affinity constants between the antibody and antigen are usually of the order of 10^8 M^{-1} and can be as high as 10^{15} M^{-1} which is significantly higher than for other biomolecules such as enzymes. Antibodies can be generated to bind to a wide range of compounds. This selective binding between antibodies and the compounds forms the basis of the detection. The main disadvantage of immunosensors is that antibodies have to be developed and characterized for each compound.

DNA is also well suited for biosensing because the base-pairing interactions between complementary sequences are both specific and robust. Typically, a single-stranded oligonucleotide probe is immobilized as a recognition material. Base-pairing interactions between the immobilized probe and its complementary target are the genesis of the transduction signal. Environmental monitoring has also benefited from different modes of DNA recognition besides base-pairing hybridization events. Unique interactions of an immobilized double-stranded DNA with low molecular weight pollutants can be utilized for detecting these substances. These interactions include (a) the preferential accumulation of pollutants by the immobilized DNA layer prior to electrochemical, optical and mass-based transduction, (b) changes in the intrinsic oxidation signal of the nucleic acid coated electrode induced by the DNA-pollutant binding and (c) detection of non-electroactive analytes *via* the competitive binding and displacement of redox markers from the surface bound DNA. Aptamers, single-stranded DNA, are another type of biological recognition element that is rapidly gaining popularity for detection of low and high molecular weight environmental pollutants. These ssDNA recognition molecules with binding affinity matching that of antibodies do not require animals and can be easily selected using high through put combinatorial techniques.

Whole-cell biosensors utilize bacteria, fungi, yeasts, animal or plant cells as recognition elements by measuring their general metabolic status. Many enzymes and co-factors that co-exist in the cells give them the ability to consume and hence detect a large number of chemicals. However, this may compromise their selectivity. Whole cells can easily be manipulated and adapted to consume and degrade new substrates.[7–9] The flexibility of whole-cell biosensors is perhaps best demonstrated by the Microtox® system. Microtox® is a standardised toxicity test system which is rapid, sensitive, and reproducible. The procedure employs the bioluminescent marine bacterium (*Vibrio fischeri*) as the test organism. The bacteria are exposed to a range of concentrations of the material being tested. The reduction in luminescence emitted from the bacteria is measured along with standard solutions and control samples.

Biosensors have proved to be extremely reliable tools in complementing and, in some cases, replacing existing analytical methodologies in the detection and monitoring of an ever increasing number of environmental contaminants. Indeed, the past three decades have witnessed a tremendous amount of activity in biosensor research and development. As a result, many review articles have been published in recent years discussing the role of biosensors in environmental analysis and monitoring. Some of these reviews have been restricted to enzyme based biosensors,[5] whole-cell biosensors,[10,11] surface plasmon resonance biosensors,[12,13] biosensors based on screen printing technologies,[14,15] biosensors for waterborne pathogens,[16] endocrine disruptors[17] or heavy metals.[18,19] Additionally, general reviews about biosensors for environmental analysis and monitoring have been published.[20–24] The aim of this review is to document recent trends and challenges in biosensor research within the last 5 years or so. Special attention will be directed to

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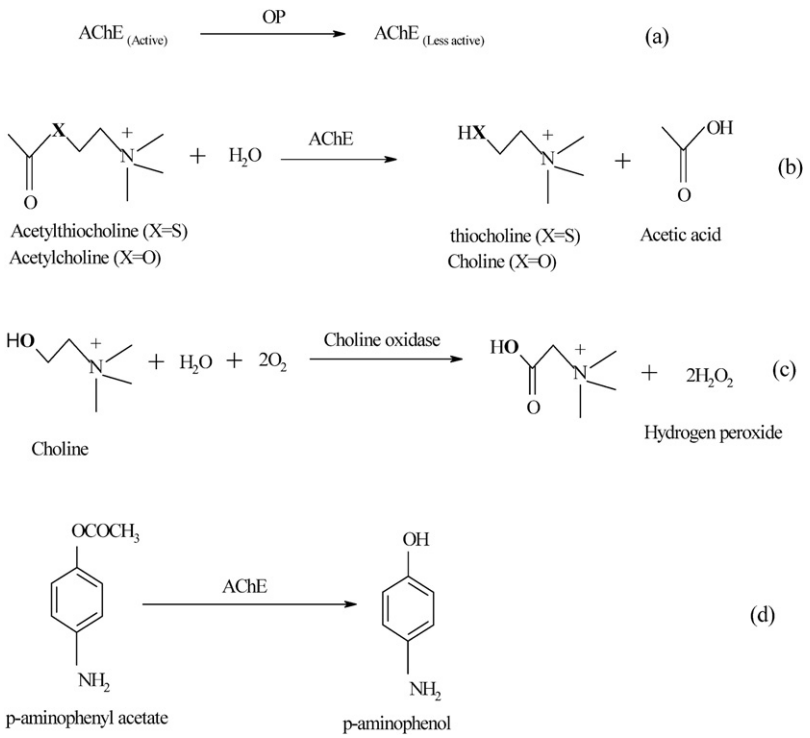
the monitoring and detection of species of environmental significance such as organophosphate nerve agents, heavy metals, organic contaminants and pathogenic microorganisms. Special focus will be given to the trends that have the most promising applications in environmental security.

Biosensors for organophosphate nerve agents

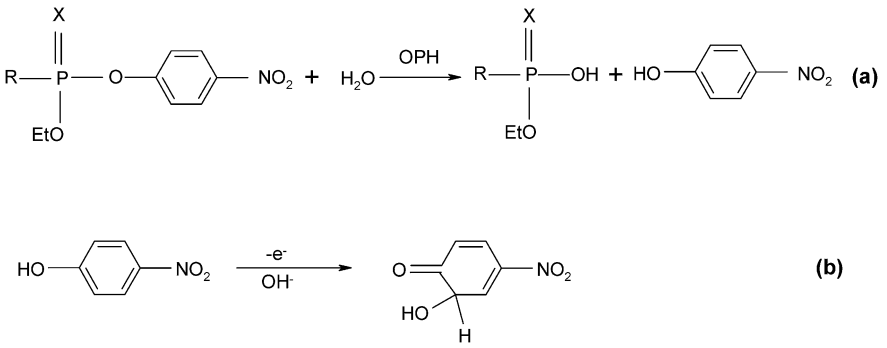
Organophosphates (OPs) were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were first discovered in 1932. Commercial compounds usually summarized under organophosphates comprise esters, amides or thiol derivatives of phosphoric, phosphonic, thiophosphoric and thiophosphonic acids. About one hundred active ingredients are or have been used in several hundred products against pests. In addition, highly toxic substances from this group have been developed as chemical warfare agents (Tabun, Soman, Sarin, VX).[25] OPs act by inhibiting the enzyme acetylcholinesterase (AChE) that is essential for normal functioning of the nervous systems. AChE plays a key role in cholinergic transmission by catalyzing the rapid hydrolysis of the neurotransmitter acetylcholine into acetate and choline.[26] The toxicity of OPs is due to their inhibition of AChE, resulting in the build up of acetylcholine which interferes with muscular responses. Signs and symptoms of OP poisoning include lacrimation, hypersalivation, bronchial hypersecretion and bronchoconstriction, urination and defecation, skeletal muscle fasciculation and twitching, ataxia, respiratory failure, convulsions, hypothermia and eventually death. Death is normally due to respiratory failure resulting from the combination of these effects.

While ELISA kits for a few OPs are commercially available, enzyme biosensors are the most widely researched sensors for the detection of OP compounds. Organophosphate hydrolase (OPH) and acetycholinesterase (AChE) are the two enzymes that have been widely applied in these enzyme biosensors for OP detection. Biosensors based on acetylcholinesterase operate by inhibition of the enzyme activity that can be measured by the detection of specific products. (Scheme 1). Detection *via* amperometry can either be done directly or by use of mediators to enhance electron transfer processes. Thus, enzyme inhibition has been measured *via* amperometric detection of thiocholine[27–34] (produced by the action of AChE on acetylthiocholine, Scheme 1b) or hydrogen peroxide[35–37] (produced by the oxidation of choline by choline oxidase, Scheme 1c) or *p*-aminophenol[38,39] (produced by hydrolysis of *p*-aminophenyl acetate by AChE, Scheme 1d). The use of p-aminophenyl has some advantages over acetylcholine and other choline substrates such as low applied potential that avoid the interferences and use of mediators resulting in a simplified protocol. Other acetylcholinesterase-based biosensors utilize potentiometric transduction by measuring the increase in pH as a result of acetic acid reduction[40–42] (Scheme 1b). In general, biosensors based on AChE inhibitions are very sensitive. However, they harbor several drawbacks. Their selectivity is limited since AChE is inhibited by neurotoxins other than OPs, such as carbamates and heavy metals to different degrees. Further, due to the irreversible nature of enzyme inhibition, they cannot be reused without regeneration of enzyme activity by reactivators such as pyridine 2-aldoxime (2-PAM).[43] Additionally, devices based on AChE generally require time-consuming multi-step protocols, are tedious and not suitable for real-time monitoring.

OPH is an organophosphostriester hydrolyzing enzyme. It has broad substrate specificity and is able to hydrolyze a number of OP pesticides such as paraoxon, parathion, coumaphos,



Scheme 1 (a) Inhibition of AChE (b) hydrolysis of acetylthiocholine/acetylcholine (c) oxidation of choline (d) hydrolysis of *p*-aminophenyl acetate



Scheme 2 (A) Reaction scheme of the OPH catalyzed hydrolysis of parathion, methyl parathion and paraoxon. (B) Electrochemical oxidation of *p*-nitrophenol. Parathion: X = S, R = ethoxy; methyl parathion: X = S, R = methoxy; paraoxon: X = O, R = ethoxy

diazinon, methyl parathion[44] *etc.*, and chemical warfare agents such as sarin and soman[45] *etc.*, The use of OPH is extremely attractive for biosensing of OPs because they act as substrates for the enzyme rather than inhibitors. OPH hydrolyses parathion, paraoxon and methylparathion to *p*-nitrophenol (Scheme 2a) which is both electroactive and chromophoric. Therefore, the *p*-nitrophenol can be detected by electrochemical (Scheme 2b) and optical methods and correlated to the amount of OP.

In general, our research group has mostly utilized electrochemical methods to detect *p*-nitrophenol (or its derivatives) and amperometry has been the preferred electrochemical method of choice.[46–52] Potentiometry has also been used to detect OPs[53–56] by measuring the protons released by the OPH catalyzed cleavage of the P–O, P–F or P–S bonds. The pH changes have been monitored by potentiometric transducers as a pH electrode,[53,54] or a field effect transistor[57] and correlated to the OP substrate concentration. Amperometry and potentiometry techniques were combined to come up with dual amperometric-potentiometric biosensors to distinguish between *p*-nitrophenol containing OPs such as parathion, methyl parathion and paraoxon from others.[58,59] The dual transduction biosensor increased the information content and minimized false positives and negatives.

OPH-modified carbon nanotubes (CNTs) were recently utilized to facilitate the detection of V-type OP nerve agents.[60] An OPH mutant with improved catalytic rate for P–S bond hydrolysis was combined with CNT for the direct, sensitive, selective and rapid amperometric detection of demeton-S and other V-type OP nerve agent simulants (Fig. 2).[60] The hydrolysis of V-type nerve agents produced thiol containing products that were detected amperometrically at the CNTs modified electrode. We also fabricated a CNT-based conductance biosensor. The CNTs were modified by OPH that hydrolyzed OPs causing real-time detectable changes in conductance of the CNTs.[61]

Optical methods have also been utilized in the detection of OPs. In this case, analysis is based on the relationship between the amount of OP hydrolyzed and the amount of chromophoric product formed as determined by absorbance measurements or other optical methods. Using this technique, we have detected paraoxon and parathion by correlating the amount of *p*-nitrophenol to the OPs.[62,63] Coumaphos was similarly detected by correlating the amount of chlorferon.[62,63] A significant advantage of optical biosensors over potentiometric biosensors in the detection of OPs is that it is possible to use a higher ionic strength

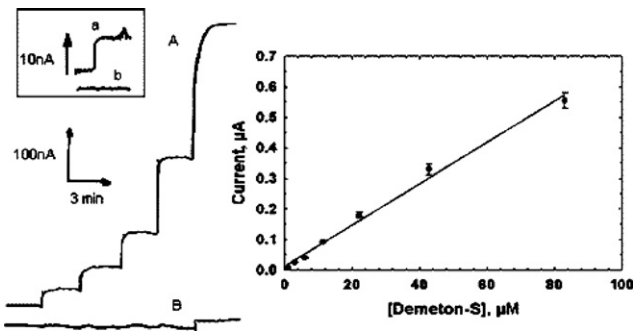


Fig. 2 Current-time traces of screen-printed electrodes modified with OPH mutant (B) and with acid-purified CNT/OPH mutant (A) to 5, 10, 20, 40, and 80 μM demeton-S. Inset shows the response of the sensor at 1 μM demeton-S with (a) and without (b) CNT. Also shown is the calibration curve for demeton-S. Measurement conditions: applied potential 0.4 V *vs.* Ag/AgCl, pH 7.4 phosphate buffer containing 0.1 M KCl. Each point represents an average of three measurements, and the error bars represent ±1 SD.[60]

buffer in the analysis in the former technique. This allows the enzyme to function at its maximum activity over the complete duration of the procedure rather than just at the start. Further, the use of ionic strength buffer eliminated the need to adjust the sample pH to that of the analytical buffer.

Recently, microcantilevers (MCLs) have emerged as novel and unique detection tool for biosensors. A biosensor based on an OPH-modified microcantilever was recently reported.[64] The OPH-modified microcantilever responded to paraoxon, parathion and diisopropyl fluorophosphates at different bending amplitude and bending rates with detection limits in the range of 10^{-7} M. The detection is most likely based on the bending as a result of conformational changes of the OPH on interaction with the substrate OPs.

Biosensors for other organic chemical contaminants

The persistence in the environment of many organic chemicals like polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and many others is of great concern because these contaminants may be accumulated through the food-chain resulting in higher concentrations in humans and other animals. PCBs have been associated with immunological abnormalities, reproductive dysfunction, increased thyroid volume and liver

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and thyroid disorders.[65] They also interfere with the endogenous hormone systems and are referred to as endocrine-disrupting chemicals (EDCs). EDCs also include compounds such as bioaccumilative organochlorines, pesticides, industrial chemicals and endogenous estrogens.

Enzyme-based biosensors have been used for the detection of phenolic estrogens. The detection principle was based on the ability of tyrosinase to catalyze the oxidation of the phenolic estrogens to *o*-diphenol and *o*-quinone. Using this principle tryosinase-carbon paste electrodes have been used for the detection of phenol,[66] catechol,[66] bisphenol A,[67] genistein,[67] quercetin,[67] nonylphenol,[67] and diethylstilbestrol[67] with detection levels in the micromolar range.

The binding of natural estrogen receptors to EDCs has been utilized to fabricate biosensors. A good example is the binding of the human estrogen receptor to bisphenol A and genistein that was recently monitored by impedance measurements.[68] Optical,[69,70] and amperometric[71] biosensors based on estrogen receptors have also been developed.

Immunosensors for PCBs were constructed by immobilizing an anti-PCB antibody within a conducting polymer matrix. The specific binding between PCB and the anti-PCB was monitored electrochemically down to ng mL^{-1} levels.[72] DNA biosensors for PCBs and aromatic amines have also been constructed. In this case, the analytical signal is the reduction of the anodic peak of guanine in the presence of increasing concentrations of the organic compounds.[73] Such lowering of the DNA intrinsic response is attributed to changes in the accessibility of the guanine moiety to the surface upon binding of the organic contaminant to the double stranded DNA.

p-Nitrophenol (PNP), a carcinogen, mutagen and cyto- and embryotoxic, is used in the manufacture of one of the most popular analgesics (acetaminophen), pesticides and dyes. It has been found in 113 of the 1416 National Priorities List sites identified by the US Environmental Protection Agency and the European Commission has set a limit of 0.1 ppb in drinking water. To selectively, sensitively and rapidly detect this compound in the field, we have utilized various bacteria to construct whole-cell biosensors.[74] Exploiting the ability of *Moraxella sp* to specifically degrade PNP to hydroquinone, a more electroactive compound than PNP, we constructed a microbial biosensor for PNP. The electrochemical oxidation current of hydroquinone was measured by a *Moraxella sp* modified carbon paste electrode and correlated to the concentration of the *p*-nitrophenol. In another microbial biosensor, we took advantage of the fact that *Moraxella sp* consumes oxygen to oxidize *p*-nitrophenol to hydroquinone. Therefore, a change in the oxygen concentration was measured by a Clark oxygen electrode and correlated to *p*-nitrophenol.[75] Similar biosensors have been fabricated with *Arthrobacter sp*.[76,77]

Heavy metal biosensors

Toxic metals, including "heavy metals," are individual metal ions and metal compounds that negatively affect people's health. Some toxic, semi-metallic elements, including arsenic and selenium, are also included in this group. In very small amounts, some of these metals are necessary to support life. However, in larger amounts, they become toxic. They may build up in biological systems and become a significant health hazard. Because of their intrinsically persistent nature, heavy metals are major contributors to pollution of the biosphere and pose major environmental security risks. Power industries, agricultural and waste disposal activities are among the leading generators of heavy metals. Many of these metals such as lead, mercury and cadmium are ranked 2nd, 3rd and 8th, respectively, according to the 2005 Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) priority list of hazardous substances in the United States. Therefore, the United States Environmental Protection Agency (US EPA), the European Commission and the United Nations have instituted very low regulatory limits for such metals. Consequently, the ability to detect and monitor these metals is crucial for our well-being.

Enzyme biosensors for detecting metal ions rely either on enzyme inhibition or activation methods. In enzyme inhibition methods, metal ions normally combine with thiol groups present in the enzyme structures thus resulting in conformational changes which affects catalytic activity. In this context, enzymes such as horseradish peroxidase,[78] alkaline phosphatase,[79] oxidases,[80–82] urease,[83–88] L-cysteine desulfhydrolase[89] and invertase[90–92] have been utilized in the detection of various metals such as arsenic, silver,[88] mercury,[2,78,81,83,88,93,94] cadmium,[2,83,87,94] lead,[88] copper,[2,81,83,88,94] and zinc.[2,88,94] However, lack of selectivity is the major disadvantage of inhibition-based enzyme biosensors as some enzymes are inhibited by several metals and even some anions and pesticides. Some researchers have attempted to alleviate this complication by the use of amino acids and peptide biomimetic ligands as recognition elements.[95–100] In some cases, very impressive results with detection limits down to picomolar levels have been realized with excellent selectivity.[99] Most of these levels are below the maximum contaminant levels in drinking water allowed by the US EPA.[101] Metal determination by enzyme activation (rather than inhibition) is much more selective because fewer metal ions can activate a particular enzyme. In this case, enzymes are only activated by specific metal ion co-factors. For example, Zn^{2+} is a necessary co-factor in the activity of alkaline phosphatase (AP) and this fact was utilized in the determination of zinc to 0.02 ppb level by the immobilization of apo-AP.[3,4] This low level of detection limit is very competitive compared to the detection limits exhibited by conventional, capital-intensive and less user-friendly instrumental techniques like graphite furnace atomic absorption spectrometry (GF-AAS) and inductively coupled plasma mass spectrometry (ICP-MS). Another advantage of enzyme activation-based methods is that the measurement is relative to a zero background signal, whereas in the enzyme inhibition-based methods high background currents make the detection of trace metals very challenging.

The discovery of catalytically active DNAs (DNA enzymes or DNAzymes) has recently led to their use in metal biosensors. DNAzymes that are highly specific for metal ions such as Pb(II), Cu(II), and Zn(II) have been obtained through a combinatorial biology approach called *in vitro* selection.[102,103] Some researchers have combined the use of a lead-specific DNAzyme with highly sensitive fluorescence detection to determine lead.[104–106] Additionally, DNAzyme-directed assembly of gold nanoparticles has recently been utilized for the extremely sensitive and selective detection of lead in paint.[107] The biosensor consisted of 5'n-thio-modified 12-mer DNA attached to 13 nm diameter gold

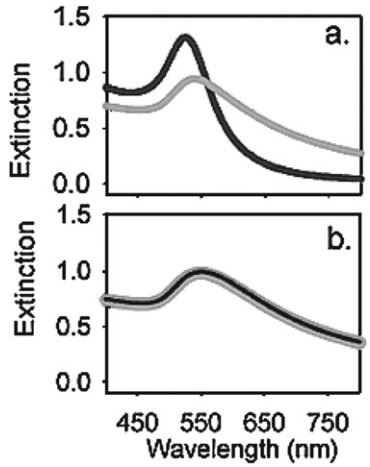

Fig. 3 UV-vis extinction spectra of an active 17E DNAzyme-nanoparticle sensor (a) and an inactive 17Ec DNAzyme-nanoparticle sensor (b) in the absence (light grey curve) or in the presence (dark grey curve) of 5 μM Pb(II).[107]

nanoparticles, a DNAzyme, and its substrate. The sequence of the substrate was designed so that it could hybridize specifically to the thiolated DNA attached to the gold nanoparticles on each end, while maintaining the DNAzyme recognition portion. These hybridizations caused aggregation of gold nanoparticles and resulted in a blue color. However, in the presence of Pb(II), the DNAzyme catalyzes hydrolytic cleavage of the substrate and prevents the formation of nanoparticle aggregates. A red color appeared as a result (Fig. 3).

Whole cell-based heavy metal sensors exploit cells that can survive in concentrated heavy metal environments. The promoters from these heavy metal resistant bacteria have been fused to various reporter genes such as *lux*, *luc*, *gfp*, and *lac Z* that express bacterial luciferase, firefly luciferase, green fluorescent protein and β-gal, respectively, to construct highly sensitive and selective bacterial biosensors. The presence of metal ion causes expression of a reporter gene that gives a signal.[108,109] Mercury,[110–112] copper,[110] lead,[113] arsenite,[114] cobalt,[115] nickel,[115] and cadmium[110,113,116] are some of the metals that have been detected in the nanomolar and even femtomolar levels using bacteria-based biosensors. These detection limits are either comparable to or lower than those exhibited by more conventional techniques like GF-AAS, ICP-MS and anodic stripping voltammetry. In general, whole cell-based biosensors are very robust and tolerant to assay conditions compared to enzymes. Further, they are self replicating and most require only the effector to elicit a response. Their limitations include maintenance of their environment with nutrients, oxygen *etc*., long response and poor batch-to-batch variability between cultures.

Biosensors for microorganisms and their toxins

In recent years, biosensors have been part of technological innovations that have resulted in more rapid, selective and sensitive detection and identification of microorganisms, viruses and their products. DNA biosensors for microorganism detection can be more specific than immunosensors and the sensitivity can be improved by combination with polymerase chain reaction (PCR) methods.[117,118] However, immunosensors are faster and more robust than DNA based detection and can simultaneously detect microorganisms and the toxins that they produce. For example, a multianalyte fluorescence based array immunosensor that was capable of simultaneously identifying bacteria, viruses and toxin proteins was recently developed. Thus, *Bacillus globigii*, MS2 bacteriopage and Staphylococcal enterotoxin were detected at 10^5 colony forming units mL^{-1}, 10^7 plaque forming units mL^{-1} and 10 ng mL^{-1}, respectively.[119] The same technology was extended to detect six different biohazards including ricin, cholera toxin, *F. tularensis* VLS, abortus, *B. anthracis* Sterne and *Staphylococcus* enterotoxin B.[120] (Fig. 4). Ebola virus was recently detected by a conducting polymer-based immunosensor.[121] The poly(pyrrole-benzophenone) film was deposited upon an indium tin oxide (ITO) modified conductive surface fiber-optic (Fig. 5). It was then linked to an Ebola virus Antigen and tested with Ebola virus by use of a coupled chemiluminescent reaction. The immunosensor was 24 times more sensitive compared to ELISA.

Recently, a new DNA biosensor for the detection of 16S rDNA, a 1500 base-pair DNA amplified from *E. coli*, was demonstrated.[122] This was done without any pre-treatment. A DNA probe was immobilized on an air plasma-activated fullerene-impregnated screen printed electrode. This resulted in an improvement in the surface coverage of the immobilized probe DNA enabling the detection of two base mismatches in 1500 base-pair DNA.

An electrochemical DNA biosensor for the detection of genes related to *Microcystis sp* (a cyanobacteria) was fabricated by the immobilization of a 17-mer DNA probe, which is complementary to a specific gene related to *Microcystis sp*.[123] The DNA probe was used to determine the amount of target gene in

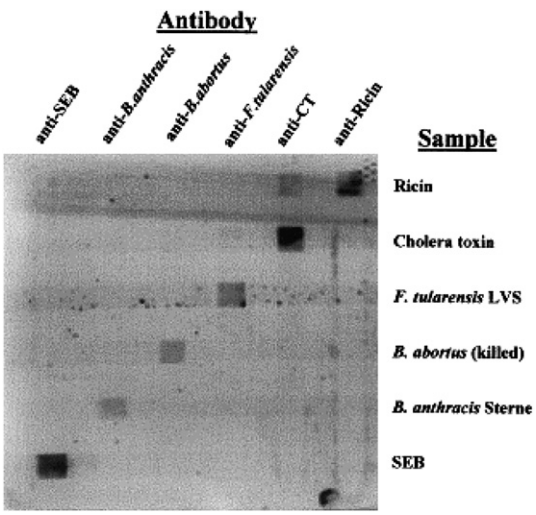

Fig. 4 Simultaneous detection of six biohazardous agents on a single sensing array. A NeutrAvidin-coated slide was patterned with columns of six 'capture' antibodies (noted above image). Six samples containing a single biohazardous analyte (indicated to the right of the image) were assayed simultaneously as described using a fluorescent tracer cocktail for detection. Concentrations of analytes were as follows: 200 ng mL^{-1} ricin, 100 ng mL^{-1} CT, 7.3×10^6 cfu mL^{-1} *F. tularensis* LVS, 1.5×10^5 cfu mL^{-1} killed *B. abortus*, 7.1×10^4 cfu mL^{-1} *B. anthracis* Sterne strain, and 100 ng mL^{-1} SEB.[120]

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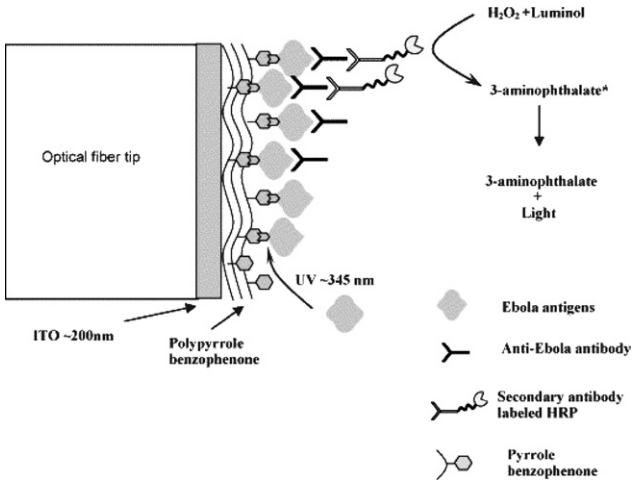

Fig. 5 The biosensor scheme describing the various steps involved in the immunoassay using ITO–poly(pyrrole-benzophenone)-coated optical fibers for the detection of anti-Ebola virus in sera samples.[121]

solution using methylene blue and ruthenium bipyridine as the electrochemical indicators. The detection limit using this approach was 90 pM. In addition, the biosensor was capable of selectively discriminating against mismatches; a very desirable condition for the detection of disease-related point-mutation in guanine bases of the cyanobacteria. Quantitative detection of genus *Microcystis* has been reported using competitive PCR followed by sequence-specific labeling of oligonucleotide probes, with a detection limit of 100 cells mL^{-1}.[124] It is evident that the biosensor allows the identification and quantification of a specific gene that is related to *Microcystis sp* in a simpler and convenient way without the need for cell culture and the PCR amplification process. A biosensor for microcystins, non-ribosomal proteins produced by *Microcystis sp*, that are considered very dangerous hepatotoxins, was recently fabricated.[125] The biosensor was fabricated on the basis of the competitive binding between the native microcystin and its fluorescent analog at immobilized alkaline phosphatase enzymes.[125] This biosensor detected microcystin down to 15 ng L^{-1} which is very much less than the 1 μg L^{-1} maximum limit of microcystin allowed by the World Health Organization Standards.[126]

Recently, nanomaterials have been utilized to lower the detection limits in microorganism sensing using DNA biosensors.[127] Nanoscale zinc oxide structures were used for the identification of the biothreat agent, *Bacillus anthracis* by successfully discriminating its DNA sequence from other genetically related species. (Fig. 6) The presence of the underlying zinc oxide nanomaterials was critical in achieving increased fluorescence detection of hybridized DNA and, therefore, accomplishing rapid and extremely sensitive identification of the microorganism.

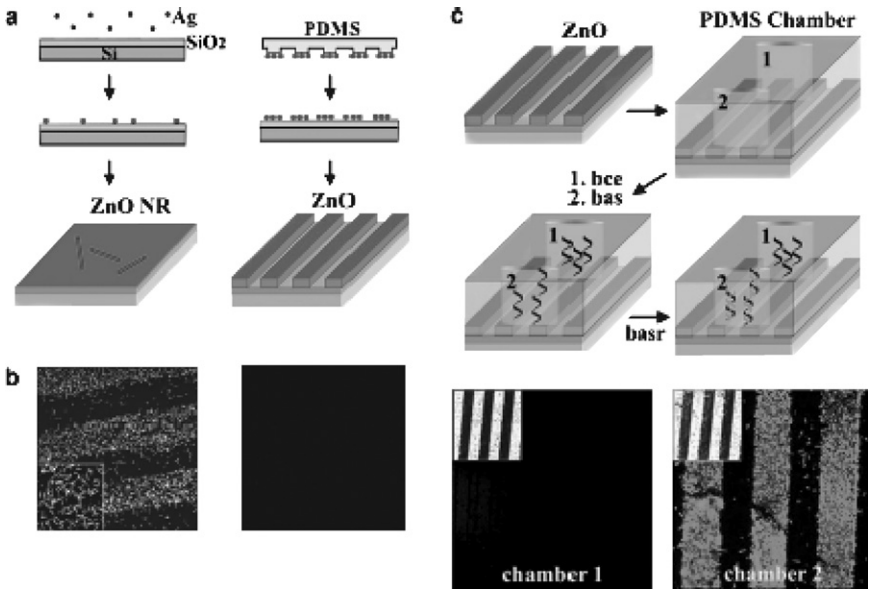

Fig. 6 (a) Schematic illustrations showing simultaneous synthesis and assembly of ZnO nanoplatforms consisting of (left) individual ZnO nanorods and (right) periodically patterned ZnO nanostructures. (b) (Left) SEM image of a patterned ZnO platform with the stripe width and repeat spacing of 50 μm. The inserted SEM image at the bottom left corner shows the lying-down arrangement of ZnO nanostructures inside the patterned stripes. (Right) Confocal fluorescence image taken from the as-synthesized, striped, ZnO nanoplatform where no fluorescence emission was detected. (c) Detection scheme to identify *B. anthracis* from *B. cereus* using ZnO nanoplatforms: PDMS chambers were used in order to carry out simultaneous hybridization reactions on the same ZnO nanoplatform. The ZnO nanoplatform contained regularly patterned ZnO stripes with a repeat spacing of 20 μm. Oligonucleotide probes of 5′-GTTACGGAAA GAACCA-3′ (*bce*) and 5′-AGTGCGCGAGGAGCCT-3′ (*bas*), were first introduced to the reaction chambers 1 and 2, respectively. Subsequently, fluorescein modified *basr* strands were added to both chambers and allowed to form DNA duplex under the same hybridization conditions. Confocal images taken from these samples showed clear fluorescence emission from chamber 2, in contrast to no discernable fluorescence signal from chamber 1. The insets in the upper left corners of the confocal images are the corresponding bright field images taken from each chamber after the duplex formation reaction. Distinctive fluorescence emission monitored from chamber 2 is due to DNA duplex formation between fully complementary strands of *bas* and 5′-TCACGCGCTCCTCGGA-3′ (*basr*), whereas the lack of duplex formation between mismatching sequences of *bce* and *basr* led to no observable fluorescence in chamber 1. The striped patterns of fluorescence emission observed from chamber 2 faithfully mimic the underlying geometry of the ZnO nanoplatform.[127]

Conclusions and future perspectives

Environmental security needs technologies that are sensitive, selective, affordable, user-friendly, portable and that consume minimal power. While most environmental biosensors address some of these requirements, further research is required to address several drawbacks associated with some biosensors. For example, some cannot be used directly in the field without extensive sample preparation. Others are time consuming or suffer from poor selectivity especially in complex matrices. Most biosensors have shown excellent characteristics for synthetic samples, but are not as effective at providing reliable information in more complex matrices mainly due to interfering compounds with similar characteristics. Further, biosensors have limited lifetimes and cannot withstand harsh conditions because of the sensitive nature of the biological material that are used in the sensor design.[128–130]

Extensive research is currently underway to alleviate some of these challenges. For example, advances in genetic engineering have, and will continue to produce novel and more selective bioreceptors that should improve the analytical performance of these devices. A good case is the increase in inhibition sensitivity by using genetically modified AChE in biosensors.[131–134] Novel gene fusions have resulted in more sensitive and versatile reporters such as GFP.[135] Particular attention should be given to mismatch discrimination and signal amplification in DNA biosensors. Peptide nucleic acid (PNA) has demonstrated remarkable hybridization properties towards complementary oligonucleotides and can be used as recognition elements in PNA-based biosensors.[136,137] On the same note, aptamers (synthetic nucleic acids) are novel recognition elements that are able to bind a wide range of target molecules with high affinity and specificity in a manner similar to antibodies.[138–140] Further, molecularly imprinted polymers (MIPs) are fast becoming an important class of synthetic materials mimicking molecular recognition by natural receptors.[141] Finally, the current trend towards miniaturization and the development of biosensors based on microfluidic platforms[142] should also enable the design of integrated systems of arrays of enzymes, antibodies and oligonucleotides that should enable the simultaneous detection of multiple analytes.

References

1 L. C. Clark and C. Lyons, *Ann. N. Y. Acad. Sci.*, 1962, **102**, 29.
2 I. Bontidean, J. R. Lloyd, J. L. Hobman, J. R. Wilson, E. Csoregi, B. Mattiasson and N. L. Brown, *J. Inorg. Biochem.*, 2000, **79**, 225.
3 S. D. Kamtekar, R. Pande, M. S. Ayyagari, K. A. Marx, D. L. Kaplan, J. Kumar and S. Tripathy, *Anal. Chem.*, 1996, **68**, 216.
4 S. D. Kamtekar, R. Pande, M. S. Ayyagari, K. A. Marx, D. L. Kaplan, J. Kumar and S. K. Tripathy, *Mater. Sci. Eng., C*, 1995, **3**, 79.
5 A. Amine, H. Mohammadi, I. Bourais and G. Palleschi, *Biosens. Bioelectron.*, 2006, **21**, 1405.
6 S. Sole, A. Merkoci and S. Alegret, *Crit. Rev. Anal. Chem.*, 2003, **33**, 127.
7 R. K. Jain, J. H. Dreisbach and J. C. Spain, *Appl. Environ. Microbiol.*, 1994, **60**, 3030.
8 K. T. Leung, O. Tresse, D. Errampalli, H. Lee and J. T. Trevors, *FEMS Microbiol. Lett.*, 1997, **155**, 107.
9 J. C. Spain and S. F. Nishino, *Appl. Environ. Microbiol.*, 1987, **53**, 1010.
10 K. Yagi, *Appl. Microbiol. Biotechnol.*, 2007, **73**, 1251.
11 Y. Lei, W. Chen and A. Mulchandani, *Anal. Chim. Acta*, 2006, **568**, 200.
12 A. A. Bergwerff and F. Van Knapen, *J. AOAC Int.*, 2006, **89**, 826.
13 D. R. Shankaran, K. V. A. Gobi and N. Miura, *Sens. Actuators, B*, 2007, **121**, 158.
14 M. Tudorache and C. Bala, *Anal. Bioanal. Chem.*, 2007, **388**, 565.
15 J. P. Hart, A. Crew, E. Crouch, K. C. Honeychurch and R. M. Pemberton, *Anal. Lett.*, 2004, **37**, 789.
16 A. Rasooly and K. E. Herold, *J. AOAC Int.*, 2006, **89**, 873.
17 S. Rodriguez-Mozaz, M. P. Marco, M. J. L. de Alda and D. Barcelo, *Anal. Bioanal. Chem.*, 2004, **378**, 588.
18 N. Verma and M. Singh, *Biometals*, 2005, **18**, 121.
19 J. J. Gooding, E. Chow and R. Finlayson, *Aust. J. Chem.*, 2003, **56**, 159.
20 S. Andreescu and O. A. Sadik, *Pure Appl. Chem.*, 2004, **76**, 861.
21 A. J. Baeumner, *Anal. Bioanal. Chem.y*, 2003, **377**, 434.
22 S. Rodriguez-Mozaz, M. J. L. de Alda and D. Barcelo, *Anal. Bioanal. Chem.*, 2006, **386**, 1025.
23 K. R. Rogers, *Anal. Chim. Acta*, 2006, **568**, 222.
24 O. A. Sadik, A. K. Wanekaya and S. Andreescu, *J. Environ. Monit.*, 2004, **6**, 513.
25 D. J. Ecobichon, Toxic Effects of Pesticides, in *Toxicology, the Basic Science of Poisons*, ed. D. J. Casaretti L, Mc Graw-Hill, 1991.
26 T. L. Rosenberry, *Adv. Enzymol. Relat. Area Mol. Biol.*, 1975, **43**, 103.
27 G. S. Nunes, G. Jeanty and J. L. Marty, *Anal. Chim. Acta*, 2004, **523**, 107.
28 T. Noguer, B. Leca, G. Jeanty and J. L. Marty, *Field Anal. Chem. Technol.*, 1999, **3**, 171.
29 S. Andreescu, L. Barthelmebs and J. L. Marty, *Anal. Chim. Acta*, 2002, **464**, 171.
30 T. T. Bachmann, B. Leca, F. Vilatte, J. L. Marty, D. Fournier and R. D. Schmid, *Biosens. Bioelectron.*, 2000, **15**, 193.
31 G. S. Nunes, D. Barcelo, B. S. Grabaric, J. M. Diaz-Cruz and M. L. Ribeiro, *Anal. Chim. Acta*, 1999, **399**, 37.
32 M. Khayyami, M. T. P. Pita, N. P. Garcia, G. Johansson, B. Danielsson and P. O. Larsson, *Talanta*, 1998, **45**, 557.
33 G. Jeanty and J. L. Marty, *Biosens. Bioelectron.*, 1998, **13**, 213.
34 K. A. Joshi, J. Tang, R. Haddon, J. Wang, W. Chen and A. Mulchandani, *Electroanalysis*, 2005, **17**, 54.
35 Y. H. Lin, F. Lu and J. Wang, *Electroanalysis*, 2004, **16**, 145.
36 F. N. Kok and V. Hasirci, *Biosens. Bioelectron.*, 2004, **19**, 661.
37 A. Guerrieri and F. Palmisano, *Anal. Chem.*, 2001, **73**, 2875.
38 S. Andreescu, A. Avramescu, C. Bala, V. Magearu and J. L. Marty, *Anal. Bioanal. Chem.*, 2002, **374**, 39.
39 S. Andreescu, T. Noguer, V. Magearu and J. L. Marty, *Talanta*, 2002, **57**, 169.
40 A. N. Ivanov, G. A. Evtugyn, R. E. Gyurcsanyi, K. Toth and H. C. Budnikov, *Anal. Chim. Acta*, 2000, **404**, 55.
41 H. S. Lee, Y. A. Kim, Y. A. Cho and Y. T. Lee, *Chemosphere*, 2002, **46**, 571.
42 K. Reybier, S. Zairi, N. Jaffrezic-Renault and B. Fahys, *Talanta*, 2002, **56**, 1015.
43 D. J. Ecobichon, in *Toxic effects of pesticides*, ed. M. O. Amdur, J. Doull, C. D. Klaasen, New York, NY, 1996.
44 D. M. Munnecke, *J. Agric. Food Chem.*, 1980, **28**, 105.
45 D. P. Dumas, H. D. Durst, W. G. Landis, F. M. Raushel and J. R. Wild, *Arch. Biochem. Biophys.*, 1990, **277**, 155.
46 P. Mulchandani, W. Chen and A. Mulchandani, *Environ. Sci. Technol.*, 2001, **35**, 2562.
47 Y. Lei, P. Mulchandani, J. Wang, W. Chen and A. Mulchandani, *Environ. Sci. Technol.*, 2005, **39**, 8853.
48 R. P. Deo, J. Wang, I. Block, A. Mulchandani, K. A. Joshi, M. Trojanowicz, F. Scholz, W. Chen and Y. H. Lin, *Anal. Chim. Acta*, 2005, **530**, 185.
49 P. Mulchandani, W. Chen, A. Mulchandani, J. Wang and L. Chen, *Biosens. Bioelectron.*, 2001, **16**, 433.
50 J. Wang, M. Pumera, M. P. Chatrathi, A. Escarpa, M. Musameh, G. Collins, A. Mulchandani, Y. Lin and K. Olsen, *Anal. Chem.*, 2002, **74**, 1187.
51 A. Mulchandani, P. Mulchandani, W. Chen, J. Wang and L. Chen, *Anal. Chem.*, 1999, **71**, 2246.
52 Y. Lei, P. Mulchandani, W. Chen and A. Mulchandani, *Appl. Biochem. Biotechnol.*, 2007, **136**, 243.
53 P. Mulchandani, A. Mulchandani, I. Kaneva and W. Chen, *Biosens. Bioelectron.*, 1999, **14**, 77.

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54 A. Mulchandani, P. Mulchandani, I. Kaneva and W. Chen, *Anal. Chem.*, 1998, **70**, 4140.
55 S. Gaberlein, F. Spener and C. Zaborosch, *Appl. Microbiol. Biotechnol.*, 2000, **54**, 652.
56 M. J. Schoning, M. Arzdorf, P. Mulchandani, W. Chen and A. Mulchandani, *Sens. Actuators, B*, 2003, **91**, 92.
57 A. L. Simonian, A. W. Flounders and J. R. Wild, *Electroanalysis*, 2004, **16**, 1896.
58 J. Wang, R. Krause, K. Block, M. Musameh, A. Mulchandani, P. Mulchandani, W. Chen and M. J. Schoning, *Anal. Chim. Acta*, 2002, **469**, 197.
59 M. J. Schoning, R. Krause, K. Block, M. Musahmeh, A. Mulchandani and J. Wang, *Sens. Actuators, B*, 2003, **95**, 291.
60 K. A. Joshi, M. Prouza, M. Kum, J. Wang, J. Tang, R. Haddon, W. Chen and A. Mulchandani, *Anal. Chem.*, 2006, **78**, 331.
61 N. Y. Liu, X. P. Cai, Y. Lei, Q. Zhang, M. B. Chan-Park, C. M. Li, W. Chen and A. Mulchandani, *Electroanalysis*, 2007, **19**, 616.
62 A. Mulchandani, I. Kaneva and W. Chen, *Anal. Chem.*, 1998, **70**, 5042.
63 A. Mulchandani, S. T. Pan and W. Chen, *Biotechnol. Prog.*, 1999, **15**, 130.
64 C. Karnati, H. W. Du, H. F. Ji, X. H. Xu, Y. Lvov, A. Mulchandani, P. Mulchandani and W. Chen, *Biosens. Bioelectron.*, 2007, **22**, 2636.
65 P. Langer, A. Kocan, M. Tajtakova, J. Petrik, J. Chovancova, B. Drobna, S. Jursa, M. Pavuk, J. Koska, T. Trnovec, E. Sebokova and I. Klimes, *J. Occup. Environ. Med.*, 2003, **45**, 526.
66 K. R. Rogers, J. Y. Becker and J. Cembrano, *Electrochim. Acta*, 2000, **45**, 4373.
67 S. Andreescu and O. A. Sadik, *Anal. Chem.*, 2004, **76**, 552.
68 V. Granek and J. Rishpon, *Environ. Sci. Technol.*, 2002, **36**, 1574.
69 E. Wozei, S. W. Hermanowicz and H. Y. N. Holman, *Biosens. Bioelectron.*, 2006, **21**, 1654.
70 T. Hahn, K. Tag, K. Riedel, S. Uhlig, K. Baronian, G. Gellissen and G. Kunze, *Biosens. Bioelectron.*, 2006, **21**, 2078.
71 E. Dempsey, D. Diamond and A. Collier, *Biosens. Bioelectron.*, 2004, **20**, 367.
72 S. Bender and O. A. Sadik, *Environ. Sci. Technol.*, 1998, **32**, 788.
73 G. Marrazza, I. Chianella and M. Mascini, *Anal. Chim. Acta*, 1999, **387**, 297.
74 P. Mulchandani, C. M. Hangarter, Y. Lei, W. Chen and A. Mulchandani, *Biosens. Bioelectron.*, 2005, **21**, 523.
75 P. Mulchandani, Y. Lei, W. Chen, J. Wang and A. Mulchandani, *Anal. Chim. Acta*, 2002, **470**, 79.
76 Y. Lei, P. Mulchandani, W. Chen, J. Wang and A. Mulchandani, *Electroanalysis*, 2004, **16**, 2030.
77 Y. Lei, P. Mulchandani, W. Chen, J. Wang and A. Mulchandani, *Electroanalysis*, 2003, **15**, 1160.
78 S. B. Han, M. Zhu, Z. B. Yuan and X. Li, *Biosens. Bioelectron.*, 2001, **16**, 9.
79 I. A. Veselova and T. N. Shekhovtsova, *Anal. Chim. Acta*, 2000, **413**, 95.
80 N. F. Starodub, N. I. Kanjuk, A. L. Kukla and Y. M. Shirshov, *Anal. Chim. Acta*, 1999, **385**, 461.
81 C. Malitesta and M. R. Guascito, *Biosens. Bioelectron.*, 2005, **20**, 1643.
82 P. W. Alexander and G. A. Rechnitz, *Electroanalysis*, 2000, **12**, 343.
83 H. C. Tsai and R. A. Doong, *Biosens. Bioelectron.*, 2005, **20**, 1796.
84 T. K. V. Krawczyk, T. Moszczynska and M. Trojanowicz, *Biosens. Bioelectron.*, 2000, **15**, 681.
85 S. Komaba, Y. Fujino, T. Matsuda, T. Osaka and I. Satoh, *Sens. Actuators, B*, 1998, **52**, 78.
86 C. Preininger, *Mikrochim. Acta*, 1999, **130**, 209.
87 L. M. May and D. A. Russell, *Anal. Chim. Acta*, 2003, **500**, 119.
88 B. Kuswandi, *Anal. Bioanal. Chem.*, 2003, **376**, 1104.
89 N. V. Kremleva, E. P. Medyantseva, G. K. Budnikov and Y. I. Bormotova, *J. Anal. Chem.*, 1999, **54**, 151.
90 H. Mohammadi, A. Amine, A. Ouarzane and M. El Rhazi, *Microchim. Acta*, 2005, **149**, 251.
91 H. Mohammadi, A. Amine, M. El Rhazi and C. M. A. Brett, *Talanta*, 2004, **62**, 951.
92 H. Mohammadi, M. El Rhazi, A. Amine, A. M. O. Brett and C. M. A. Brett, *Analyst*, 2002, **127**, 1088.
93 R. A. Doong and H. C. Tsai, *Anal. Chim. Acta*, 2001, **434**, 239.
94 I. Bontidean, C. Berggren, G. Johansson, E. Csoregi, B. Mattiasson, J. A. Lloyd, K. J. Jakeman and N. L. Brown, *Anal. Chem.*, 1998, **70**, 4162.
95 E. Chow, D. Ebrahimi, J. J. Gooding and D. B. Hibbert, *Analyst*, 2006, **131**, 1051.
96 E. Chow, D. B. Hibbert and J. J. Gooding, *Analyst*, 2005, **130**, 831.
97 E. Chow, D. B. Hibbert and J. J. Gooding, *Electrochem. Commun.*, 2005, **7**, 101.
98 W. R. Yang, J. J. Gooding and D. B. Hibbert, *J. Electroanal. Chem.*, 2001, **516**, 10.
99 W. R. Yang, D. Jaramillo, J. J. Gooding, D. B. Hibbert, R. Zhang, G. D. Willett and K. J. Fisher, *Chem. Commun.*, 2001, 1982.
100 W. R. Yang, J. J. Gooding and D. B. Hibbert, *Analyst*, 2001, **126**, 1573.
101 USEPA, *United States EPA, EPA 816-F-03-016*, 2003.
102 R. R. Breaker, *Curr. Opin. Biotechnol.*, 2002, **13**, 31.
103 R. R. Breaker, *Chem. Rev.*, 1997, **97**, 371.
104 J. Li and Y. Lu, *J. Am. Chem. Soc.*, 2000, **122**, 10466.
105 I. H. Chang, J. J. Tulock, J. W. Liu, W. S. Kim, D. M. Cannon, Y. Lu, P. W. Bohn, J. V. Sweedler and D. M. Cropek, *Environ. Sci. Technol.*, 2005, **39**, 3756.
106 Y. Xiao, A. A. Rowe and K. W. Plaxco, *J. Am. Chem. Soc.*, 2007, **129**, 262.
107 J. W. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2003, **125**, 6642.
108 C. Rensing and R. M. Maier, *Ecotoxicol. Environ. Saf.*, 2003, **56**, 140.
109 S. Belkin, *Curr. Opin. Microbiol.*, 2003, **6**, 206.
110 P. Corbisier, D. van der Lelie, B. Borremans, A. Provoost, V. de Lorenzo, N. L. Brown, J. R. Lloyd, J. L. Hobman, E. Csoregi, G. Johansson and B. Mattiasson, *Anal. Chim. Acta*, 1999, **387**, 235.
111 A. Roda, P. Pasini, N. Mirasoli, M. Guardigli, C. Russo, M. Musiani and M. Baraldini, *Anal. Lett.*, 2001, **34**, 29.
112 L. D. Rasmussen, S. J. Sorensen, R. R. Turner and T. Barkay, *Soil Biol. Biochem.*, 2000, **32**, 639.
113 S. Tauriainen, M. Karp, W. Chang and M. Virta, *Biosens. Bioelectron.*, 1998, **13**, 931.
114 H. Fujimoto, M. Wakabayashi, H. Yamashiro, I. Maeda, K. Isoda, M. Kondoh, M. Kawase, H. Miyasaka and K. Yagi, *Appl. Microbiol. Biotechnol.*, 2006, **73**, 332.
115 C. Tibazarwa, P. Corbisier, M. Mench, A. Bossus, P. Solda, M. Mergeay, L. Wyns and D. van der Lelie, *Environ. Pollut.*, 2001, **113**, 19.
116 V. Prachayasittikul, C. I. N. Ayudhya and L. Bulow, *Biotechnol. Lett.*, 2001, **23**, 1285.
117 C. A. Bell, J. R. Uhl, T. L. Hadfield, J. C. David, R. F. Meyer, T. F. Smith and F. R. Cockerill, *J. Clin. Microbiol.*, 2002, **40**, 2897.
118 G. A. Tipples, D. Safronetz and M. Gray, *J. Virol. Methods*, 2003, **113**, 113.
119 C. A. Rowe, L. M. Tender, M. J. Feldstein, J. P. Golden, S. B. Scruggs, B. D. MacCraith, J. J. Cras and F. S. Ligler, *Anal. Chem.*, 1999, **71**, 3846.
120 C. A. Rowe-Taitt, J. W. Hazzard, K. E. Hoffman, J. J. Cras, J. P. Golden and F. S. Ligler, *Biosens. Bioelectron.*, 2000, **15**, 579.
121 A. Petrosova, T. Konry, S. Cosnier, I. Trakht, J. Lutwama, E. Rwaguma, A. Chepurnov, E. Muhlberger, L. Lobel and R. S. Marks, *Sens. Actuators, B*, 2007, **122**, 578.
122 H. Shiraishi, T. Itoh, H. Hayashi, K. Takagi, M. Sakane, T. Mori and J. Wang, *Bioelectrochemistry*, 2007, **70**, 481.
123 F. Yan, A. Erdem, B. Meric, K. Kerman, M. Ozsoz and O. A. Sadik, *Electrochem. Commun.*, 2001, **3**, 224.
124 K. Rudi, O. M. Skulberg, F. Larsen and K. S. Jakobsen, *Appl. Environ. Microbiol.*, 1998, **64**, 2639.
125 O. A. Sadik and F. Yan, *Chem. Commun.*, 2004, 1136.
126 WHO, 1998.
127 N. Kumar, A. Dorfman and J. Hahm, *Nanotechnology*, 2006, **17**, 2875.
128 M. Campas, B. Prieto-Simon and J. L. Marty, *Talanta*, 2007, **72**, 884.
129 S. Andreescu and J. L. Marty, *Biomol. Eng.*, 2006, **23**, 1.
130 P. Leonard, S. Hearty, J. Brennan, L. Dunne, J. Quinn, T. Chakraborty and R. O'Kennedy, *Enzyme Microb. Technol.*, 2003, **32**, 3.
131 G. Istarnboulie, S. Andreescu, J. L. Marty and T. Noguer, *Biosens. Bioelectron.*, 2007, **23**, 506.
132 S. Sotiropoulou, D. Fournier and N. A. Chaniotakis, *Biosens. Bioelectron.s*, 2005, **20**, 2347.
133 P. Marques, G. S. Nunes, T. C. R. dos Santos, S. Andreescu and J. L. Marty, *Biosens. Bioelectron.*, 2004, **20**, 825.

134 B. Bucur, A. F. Danet and J. L. Marty, *Anal. Chim. Acta*, 2005, **530**, 1.
135 A. Muller-Taubenberger and K. I. Anderson, *Appl. Microbiol. Biotechnol.*, 2007, **77**, 1.
136 J. Wang, *Biosens. Bioelectron.*, 1998, **13**, 757.
137 K. Kroger, A. Jung, S. Reder and G. Gauglitz, *Anal. Chim. Acta*, 2002, **469**, 37.
138 S. Tombelli, M. Minunni and M. Mascini, *Biomol. Eng.*, 2007, **24**, 191.
139 C. L. A. Hamula, J. W. Guthrie, H. Q. Zhang, X. F. Li and X. C. Le, *TrAC, Trends Anal. Chem.*, 2006, **25**, 681.
140 S. Tombelli, A. Minunni and A. Mascini, *Biosens. Bioelectron.*, 2005, **20**, 2424.
141 M. C. Blanco-Lopez, M. J. Lobo-Castanon, A. J. Miranda-Ordieres and P. Tunon-Blanco, *TrAC, Trends Anal. Chem.*, 2004, **23**, 36.
142 C. Q. Yi, Q. Zhang, C. W. Li, J. Yang, J. L. Zhao and M. S. Yang, *Anal. Bioanal. Chem.*, 2006, **384**, 1259.

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