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Mini Review

Biosensors for heavy metals

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Abstract

A biosensor is an analytical device that consists of an immobilized biocomponent in conjunction with a transducer, and represents a synergistic combination of biotechnology and microelectronics. This review summarizes the use of biosensors for detecting and quantifying heavy metal ions. Heavy metal contamination is of serious concern to human health since these substances are non-biodegradable and retained by the ecological system. Conventional analytical techniques for heavy metals (such as cold vapour atomic absorption spectrometry, and inductively coupled plasma mass spectrometry) are precise but suffer from the disadvantages of high cost, the need for trained personnel and the fact that they are mostly laboratory bound. Biosensors have the advantages of specificity, low cost, ease of use, portability and the ability to furnish continuous real time signals. The analysis of heavy metal ions can be carried out with biosensors by using both protein (enzyme, metal-binding protein and antibody)-based and whole-cell (natural and genetically engineered microorganism)-based approaches.

Introduction

Biosensors – a synergistic combination of biotechnology and microelectronics

A biosensor is an analytical device that consists of an immobilized biological material in intimate contact with a compatible transducer, which will convert the biochemical signal into a quantifiable electrical signal (Gronow 1984). Biosensors are the offspring of the first successful marriage between biotechnology and modern electronics. The biomolecules are responsible for the specific recognition of the analyte whereas the physicochemical transducer supplies an electrical output signal which is amplified by the electronic component (Scheller & Schubert 1992). The specificity of enzymes is the main reason for their use in biosensors. Since most of the enzymes employed for use in sensors have been isolated from microorganisms, it is logical that the organisms themselves should be regarded as potential biocatalysts (Aston & Turner 1984). In microorganisms, the enzymes remain in their natural environment, increasing stability and activity (Guilbault 1984; Corcoran & Rechnitz 1985; Luong *et al.* 1988; D'Souza 2001; Verma & Singh 2003). Cell membranes and organelles can also be used for biosensor construction (Burstein *et al.* 1986, Verma & Malaku 2001). Specific binding between antibody and antigen can be exploited in immunobiosensors. To detect very low concentrations of substances such as drugs, toxins or explosives, receptor-based sensors are very appealing (Prasad *et al.* 2004).

Various immobilization procedures have been used in biosensor construction. In general, the choice of procedure depends on the nature of the biological element, the type of transducer used, the physicochemical properties of the analyte and

the operating conditions in which the biosensor is to function. Perhaps over-riding all the considerations is the necessity for the biological component to exhibit high activity with appropriate specificity in its immobilized microenvironment. The four main approaches to enzyme and microbial immobilization are entrapment and encapsulation, covalent binding, cross linking and adsorption (Brodelius & Vandamme 1987; Kennedy & Cabral 1987; Luong *et al.* 1988; Scheller & Schubert 1992).

Several types of transducers have been used in biosensor construction such as electrochemical (amperometric, potentiometric), thermal, polarimetric, piezoelectric and surface acoustic wave, optical and field effect transducers (Turner *et al.* 1987; Luong *et al.* 1988, 1997; Yim *et al.* 1993; Togawa *et al.* 1997; Tauriainen *et al.* 1998). Some of the major attributes of a good biosensing system are its specificity, reliability, portability, (in most cases) ability to function in optically opaque solutions, real-time analysis and simplicity of operation (D'Souza 2001).

Analysis of heavy metal ions

Heavy metals are the cause of one of the most serious pollution problems of our time. Even in small concentrations, they are a threat to the environment and human health because they are non-biodegradable (Peavy *et al.* 1988). People have always been exposed to heavy metals in the environment. Metals leaching from eating utensils and cookware lead to metallic contamination of food and water. Metallic constituents of pesticides and therapeutic agents are additional sources of hazardous exposure. The burning of fossil fuels containing heavy metals, the addition of *tetra*-ethyl lead to gasoline, and the increase in industrial applications of metals, such as metal plating factories, mining industries, tanning, dye and chemical manufacturing industries, etc., have made heavy metal poisoning a major source of environmental pollution (Klaassen 1996). Lead, chromium, cadmium, copper, zinc and mercury are among the most frequently observed metal contaminants (Maffia & Davis 2001; Barondeau *et al.* 2002; Liu & Lu 2003). The recognition of toxic effects from minute concentrations of heavy metals has resulted in regulations to reduce their presence in the environment to very low levels. Consequently, environmental awareness is growing among consumers and industrialists while legal constraints on emissions both at national and international levels are becoming increasingly strict. There is a clear need for reliable, efficient and cost-effective wastewater treatment technologies and monitoring of the environment for the presence of heavy metals that adversely affect human health.

Conventional techniques to analyse metals include cold vapour atomic absorption spectrometry, inductively coupled plasma mass spectrometry, UV visible spectrophotometry and X-ray absorption spectroscopy (APHA 1995; Breuil *et al.* 1998; Tiemann *et al.* 1998; Townsend *et al.* 1998). These techniques, although highly precise, suffer from the disadvantages of high cost, the need for trained personnel and the fact that they are mostly laboratory bound. For the reasons cited earlier, biosensors are now being utilized for monitoring heavy metal concentrations (Rogers & Lin 1992; Davis *et al.* 1995; Mulchandani & Bassi 1995; D'Souza 2001). Furthermore, their biological base makes them ideal for toxicological measurement of heavy metals, while conventional techniques can only measure concentration (Dennison & Turner 1995; Riedel *et al.* 2002). The current review, emphasizing examples from 1990 onwards, focuses on biosensor analyses of heavy metals as an alternative approach to conventional analytical procedures. A broad classification of the types of biosensors used for heavy metal analysis is shown in Figure 1.

Biosensors for heavy metals

Enzyme-based biosensors

A variety of enzymes have been used in the analysis of heavy metal ions based on activation or inhibition of their activities. Heavy metals cause activation when they form an integral part of the structure and function of the enzyme as cofactors in metalloproteins. For example, a calorimetric biosensing system for flow injection microanalysis of zinc ions has been developed. The study was based on alkaline phosphatase apoenzyme reactivation by the metal cofactor, a reaction that is exothermic. The enzyme was covalently immobilized and Zn (II) was detected over the range of 10 μM–1.0 mM with a response time of 3 min. The biosensor had a long-term operational stability of

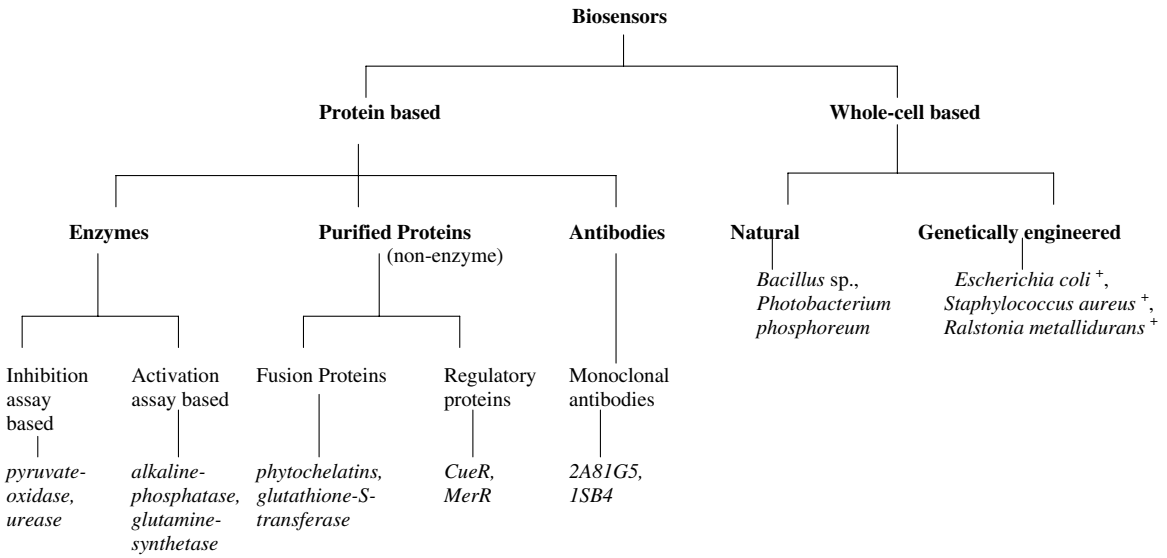

Figure 1. Classification of the types of biosensors used for the analysis of heavy metals. The specific examples cited are described in the text.

up to 2 months and was able to be regenerated in 2, 6-pryridine dicarboxylate solution (Satoh 1991).

The more common situation of heavy metal inhibition of enzymes is based, at least in part, on the interaction of metal ions with exposed thiol- or methylthiol-groups of protein amino acids (Corbisier *et al.* 1999; Krawczyk *et al.* 2000). The inhibitory action of various metal ions on enzyme oxidases and dehydrogenases illustrate this behaviour for use in biosensors. The enzymes were immobilized by reticulation in gelatin film and by covalent binding on an affinity membrane. The transducer was an amperometer with a Clark electrode. The metal ion concentration that inhibits by 50%, expressed as I_{50}, was 20 μM $HgCl_2$ for L-glycerolphosphate oxidase. The inhibition of the biocomponent was reversed by a mixture of EDTA and dithiothreitol (DTT). In a similar manner, pyruvate oxidase exhibited an I_{50} of 50 nM $HgCl_2$ with an incubation time of 2 min, and was regenerable in a buffer containing Mg^{2+} and thiamine-pyrophosphate. The authors also developed a biosensor comprised of a coupled enzyme system involving L-lactate dehydrogenase with the metal ion insensitive L-lactate oxidase, which showed an I_{50} at 1.0 μM $HgCl_2$, 0.1 μM $AgNO_3$, 10 μM $CdCl_2$, 10 μM $ZnCl_2$, 50 μM Pb $(CH_3COO)_2$ and 250 μM $CuSO_4$ upon incubation for 5 min with the enzyme. The use of different enzymes in different buffer systems allowed measurements of specific heavy metals (Gayet *et al.* 1993). The inhibitory action of Cr has also been characterized using L-lactate dehydrogenase, hexokinase and pyruvate kinase. This study involved identification of various pollutants, including metal ions, based on models constructed using artificial neural net computing technology and was therefore semi-quantitative (Cowell *et al.* 1995). A sensitive biosensor using L-lactate dehydrogenase co-immobilized with L-lactate oxidase on an oxygen electrode was developed for the determination of Hg(II), Ag(I), Pb(II), Cu(II) and Zn(II) based on lactate dehydrogenase inhibition, with detection limits of 0.002, 0.02, 0.2, 0.5 and 5.0 μM, respectively. The sensor was regenerable in 10 min with a combination of EDTA, KCN and DTT (Fennouh *et al.* 1998). The inhibition of peroxidase by mercury has been studied after immobilization in chitosan films, on polystyrene plate and on chromatography paper. Oxidation reactions of *o*-dianisidine, *o*-phenylenediamine and 3,3′, 5, 5′-tetramethylbenzidine by H_2O_2 were the indicators. The sensor had a detection limit of 0.02–1000 μM Hg (II) (Shekhovtsova *et al.* 1997).

The inhibition of urease by heavy metals has been studied by many workers. A fibre optic biosensor based on urease, immobilized on controlled pore glass, with a response time of 10–12 min has been developed for determination of mercury. The sensor had a detection range of 1.0–10 μM Hg(II)

(Andres & Narayanaswamy 1995). One of the disposable approaches was based on ammonia gas sensitive optode and an ammonium ion sensitive optode containing nonactin, an NH_4^+ selective ionophore. The efficiency of inhibition was most profound in the case of Ag(I) which had a detection limit of 0.02 ppm (0.18 μM), followed by Hg(II) and Cu(II) having detection limits of 0.07 ppm (0.35 μM) and 0.25 ppm (3.94 μM), respectively. Other metals were also investigated, but had higher limits of detection. The study also showed that the three metals mentioned had synergistic effects on inhibition, showing higher inhibition when present in combination (Preininger & Wolfbeis 1996). Another study detected heavy metal ions by inhibition of immobilized urease, with an ion selective field effect transistor (ISFET) as transducer. Using this approach, the I_{50} for Ag(I), Hg(II) and Cu(II) was 0.2, 1.5 and 5.0 μM, respectively. The biosensor was made to respond specifically towards Hg(II) by adding NaI, to suppress Ag(I) interference, and by washing in EDTA to remove Cu(II) (Nam *et al.* 1997). The inhibition of urease by mercury using a potentiometric urea biosensor was also studied. The sensor had a detection range of 0.05–1.0 μM and was regenerable in EDTA and thioacetamide; however, it showed poor long-term stability (Krawczyk *et al.* 2000). More recently, mercury, copper, cadmium and zinc inhibition of urease was examined using a coupled urease–glutamic dehydrogenase system as the biocomponent, with amperometric transduction. The lowest detectable limits of Hg(II), Cu(II), Cd(II) and Zn(II) by the biosensor are 7.2 ppm (0.035 mM), 8.5 ppm (0.13 mM), 0.3 ppm (2.67 μM) and 0.2 ppm (3.05 μM), respectively, with a response time of 15 min (Rodriguez *et al.* 2004). Table 1 shows the various enzymes that have been used as biosensors for heavy metals.

Other protein-based biosensors

A variety of non-enzymatic proteins, ranging from naturally occurring metal-binding proteins to various engineered proteins that are constructed to bind specific metal ions, have been utilized in biosensor development. A fusion of synechococcal SmtA metallothionine with glutathione-S-transferase illustrates one approach used in a biosensor. The modified cyanobacterial protein, exhibiting broad selectivity towards heavy metals, was immobilized on ethyl dimethyl aminopropyl carbodiimide and shown to bind to Zn(II), Cd(II), Cu(II) and Hg(II) with high sensitivity (i.e. fM levels). The glutathione-S-transferase-SmtA electrodes were capacitance-based, regenerated in EDTA and stable over 16 days (Corbisier *et al.* 1999). Heavy metal binding proteins like the metallothionein SmtA, regulatory protein MerR periplasmic protein MerP and the synthetic phytochelatin EC20 have been used for designing, constructing, and characterizing biosensors for the determination of various heavy metal ions such as mercury, copper, cadmium, zinc and lead, in a wide concentration range (fM–mM). The developed heavy metal biosensors had a useful storage stability (about 2 weeks) and could be regenerated using EDTA (Bontidean 2002). A capacitive transduction biosensor was developed with a synthetic phytochelatin as the sensing element, for the analysis of Hg(II), Cd(II), Pb(II), Cu(II) and Zn(II) ions in the range of 100 fM–10 mM. The sensor was regenerable in EDTA and had a storage stability of 15 days (Bontidean *et al.* 2003). *In situ* determination of Cu(II) ions has been reported using a fluorescence-based fibre optic biosensor. The biocomponent is a variant of human carbonic anhydrase II that was labelled with Oregon Green C2-iodoacetamide, a fluorophore. The conjugate was immobilized on quartz slides on the distal end of an optic fibre cable. The basis of transduction is that the metal ion binding to a site-specific apoprotein–fluorophore conjugate results in partial quenching of fluorescence. The limit of detection of the sensor was 0.1 pM Cu(II). A limitation is that the sensor loses stability after a few hours possibly due to biofouling and interference by Zn(II) or Hg(II) (Zeng *et al.* 2003).

Table 2 is a representation of purified proteins that have been used as biosensors for heavy metals.

Two examples illustrate future directions for development of protein-based biosensors. An engineered green fluorescent protein (GFP), mutant BFPms1, can be used to monitor metal binding based on alteration in fluorescence properties. Zn(II) and Cu(II) bind to this protein with different coordination geometries with Zn(II) causing enhanced fluorescence intensity and Cu(II) leading to quenching of fluorescence (Barondeau *et al.* 2002). Additional examples of proteins

Table 1. Enzymes as heavy metal biosensors.

Enzyme	Heavy metal	Lowest detectable conc. (or I_{50})	Device	Reference
Alkaline phosphatase	Zn	10 μM	Calorimeter	Satoh 1991
L-glycerolphosphate oxidase	Hg	20 μM (I_{50})	Amperometer with Clark electrode	Gayet *et al.* 1993
Pyruvate oxidase	Hg	50 nM (I_{50})	Amperometer with Clark electrode	Gayet *et al.* 1993
L-lactate dehydrogenase	Hg	1.0 μM (I_{50})	Amperometer with Clark electrode	Gayet *et al.* 1993
	Ag	0.1 μM (I_{50})		
	Cd	10 μM (I_{50})		
	Zn	10 μM (I_{50})		
	Pb	50 μM (I_{50})		
	Cu	250 μM (I_{50})		
	Hg	0.002 μM	Amperometer with O_2 electrode	Fennouh *et al.* 1998
	Ag	0.02 μM		
	Pb	0.2 μM		
	Cu	0.5 μM		
	Zn	5.0 μM		
Peroxidase	Hg	0.02 μM	Colorimeter (oxidation-based)	Shekhovtsova *et al.* 1997
Urease	Hg	1.0 μM	Fibre optic	Andres & Narayanaswamy 1995
	Ag	0.18 μM	Optical	Preininger & Wolfbeis 1996
	Hg	0.35 μM		
	Cu	3.94 μM		
	Ag	0.2 μM (I_{50})	ISFET	Nam *et al.* 1997
	Hg	1.5 μM (I_{50})		
	Cu	5.0 μM (I_{50})		
	Hg	0.05 μM	Potentiometer	Krawczyk *et al.* 2000
	Hg	0.04 mM	Amperometer	Rodriguez *et al.* 2004
	Cu	0.13 mM		
	Cd	2.67 μM		
	Zn	3.05 μM		

designed to bind metals with accompanying change in properties are likely. As an example of the potential sensitivity of the protein-based biosensors, the regulatory protein CueR, a member of the MerR family of transcriptional activators in *E. coli*, was studied for its sensitivity towards Cu(II) ions. The CueR-dependent regulation of RNA polymerase transcription at the P_{copA} promoter revealed a zeptomolar (10^{21} M) sensitivity for Cu(II) (Changela *et al.* 2003).

Antibody-based biosensors

Immunoassays have emerged as an alternate approach for metal ion detection since they offer significant advantages over traditional detection

Table 2. Purified non-enzyme proteins as heavy metal biosensors.

Protein	Heavy metal	Lowest detectable conc.	Device	Reference
Glutathione-S-transferase-SmtA	Zn Cd Cu Hg	10^{15} M	Capacitor	Corbisier *et al.* 1999
Synthetic phytochelatin	Hg Cd Pb Cu Zn	100 fM	Capacitor	Bontidean *et al.* 2003
Human carbonic anhydrase II variant	Cu	0.1 pM	Fibre optic	Zeng *et al.* 2003

methods such as high sensitivity, selectivity and species-specificity and are theoretically applicable to any pollutant for which a suitable antibody can be generated (Blake 1995). Monoclonal antibodies have been generated that recognize metal–EDTA complexes of cadmium, mercury, copper, nickel, lead, cobalt and silver, besides many other metal ions. The developed antibodies had maximum binding affinity for Cd(II) and were studied at a concentration of 100 ppm Cd–EDTA–bovine serum albumin (BSA) complex (Blake *et al.* 1996). An inhibition immunoassay that is insensitive to the presence of interfering metal ions has been employed for the analysis of cadmium using anti-cadmium (2 A8 1G5) monoclonal antibodies that bind tightly to Cd–EDTA complex but not to metal-free EDTA. These antibodies are able to detect Cd(II) in the range of 70–500 ppb (0.06–4.45 μM) (Khosraviani *et al.* 1998). Useful monoclonal antibodies have been purified that recognize zinc, cobalt and nickel–diethylenetriamine pentaacetic acid (DTPA) complexes and a monoclonal antibody that recognizes lead (II) – cyclohexyldiethylene-triamine-pentaacetic acid (CHXDTPA) (Blake *et al.* 1998). More recently, monoclonal antibodies for complexes of cadmium–EDTA, cobalt–DTPA and lead–CHXDTPA with enhanced sensitivities of 0.25, 10 and 6.0 nM, respectively have been developed (Blake *et al.* 2001). Table 3 lists the antibodies developed for various metal ions.

DNA-based biosensors

As an example of a new direction in biosensor development, a non-protein based example of a biosensor can be cited. In particular, a calorimetric Pb(II) sensor has been reported that is based on DNAzyme-directed assembly of gold nanoparticles. A "8-17" DNAzyme is the metal sensing element and shows high activity and selectivity towards Pb(II). The aggregation of the DNAzyme, its substrate and gold nanoparticles result in a blue colour; however, the presence of Pb(II) prevents the formation of nanoparticle aggregates and a red colour appears. The sensor is capable of detecting Pb(II) in the range of 100 nM–4.0 μM (Liu & Lu 2003).

Naturally occurring whole-cell based biosensors

The area of whole-cell microbial biosensors looks very promising for a multitude of uses. The response characteristics of cell-based sensors are comparable to those exhibited by enzyme electrodes. However, the microbial electrodes have certain advantages over conventional enzyme electrodes. The microbial strains are cheaper than isolated enzymes and the enzyme activity is often enhanced in microbial cells owing to optimal environment provided by the cells (Corcoron & Rechnitz 1985, D'Souza 2001). Specific metabolic pathways in microorganisms are used, resulting in the development of microbial sensors for more selective analysis of compounds or pollutants, which cannot be measured by simple enzyme reactions, e.g. the determination of aromatic compounds and heavy metals (Riedel *et al.* 2002). For example, a strain of luminous-bacterium *Photobacterium phosphoreum* MT 10204 was immobilized on a cellulose nitrate membrane filter and used to develop a microbial sensor for the determination of chromium based on a luminescence-based inhibition bioassay, achieving I_{50} at 0.85 nM Cr(VI) (Lee *et al.* 1992). Inhibition of bacterial growth in the presence of copper ions was monitored based on the change in frequency of an

Table 3. Antibodies used as heavy metal biosensors.

Antibody	Heavy metal	Lowest detectable conc.	Device	Reference
Monoclonal antibodies	Cd	100 ppm Cd–EDTA–BSA complex	Microwell ELISA format	Blake *et al.* 1996
anti-Cd monoclonal antibodies(2A81G5)	Cd	0.06 μM	Microwell ELISA format	Khosraviani *et al.* 1998
Monoclonal antibodies	Cd	0.25 nM	KinExA^{TM} Immunoassay instrument	Blake *et al.* 2001
	Co	10 nM		
	Pb	6 nM		

uncoated piezoelectric quartz crystal of a surface acoustic wave transducer, upon contact with biomass. The inhibition effect of Cu(II) on the biomass was in the range of 18.0–25.0 ppm (0.28–0.39 mM) (Yamasaki *et al.* 2004).

Genetically engineered microorganism (GEM)-based biosensors

Bacteria carrying determinants of heavy metal resistance often are specific to one or a few metals, and may utilize mechanisms of resistance that include efflux of the metal, sequestration of the metal, or a combination of these mechanisms. The proteins conferring metal resistance, or involved in the regulatory mechanisms and GEMs encoding these components have been exploited for the biomonitoring of heavy metal contaminated environments (Brown *et al.* 1998).

A recombinant bacterial strain for measuring cadmium and lead has been developed. It consists of a sensor plasmid pT 0024 that carries the firefly luciferase reporter gene under the control of the *cadA* promoter from the *cadA* resistance determinant of *Staphylococcus aureus* NCTC 50581 (p1258) expressed in *S. aureus* strain RN 4220 and *Bacillus subtilis* strain BR151. Strain RN 4220 (pT0024) responded to Cd(II) and Pb(II) with the lowest detectable limits of 10 and 33 nM, respectively. The response was obtained within 2–3 h of incubation time (Tauriainen *et al.* 1998). Similarly the soil bacterium *Alcaligenes eutrophus* (also called *Ralstonia eutropha* and now as *Ralstonia metallidurans*) is used for monitoring bioavailable heavy metal ions by making use of promoter cassettes using the *lux CDABE* reporter systems (Corbisier *et al.* 1999). The copper sensor AE 1239 exhibited a sensing range of 1–200 μM Cu(II) after an induction time of 1.5 h. The recombinant strain was immobilized in alginate and agar. The agarose immobilized strain lost 84% of its activity after 6 days whereas the alginate immobilized strain was completely stable. *A. eutrophus* AE 2440 responded to Cr(VI) in the range 1–40 μM and *A. eutropus* CH34 responded to Pb(II) in the range 0.5–8.0 μM.

The amperometric detection of metal ions using recombinant *Saccharomyces cerevisiae* strains as the biocomponent of the microbial sensor has been reported. For this purpose, plasmids were constructed containing the copper inducible promoter of the *CUP1* gene from *S. cerevisiae* which were fused to the promoterless *lac Z* gene of *E. coli*, such that the fusion construct is transcribed and translated in the presence of copper. The plasmid construct was transformed into *S. cerevisiae* and the recombinant strain immobilized with 2.5% polyvinyl alcohol on a capillary membrane. The sensor had a detection range of 0.5–2.0 mM $CuSO_4$ with a metal incubation of 20 min (Lehmann *et al.* 2000). *R. eutropha* – strain AE2515 has been developed to serve as a whole-cell biosensor for the detection of bioavailable concentrations of Ni(II) and Co(II) in soil samples. This strain is a *R. eutropha* CH34 derivative containing pMOL1550, in which the *cnrYXH* regulatory genes are transcriptionally fused to the bioluminescent *luxCDABE* reporter system. Strain AE2515 was used for its specific responses to Ni(II) and Co(II) and was able to specifically detect these metal ions in the broad range of 0.1–6.0 μM and 9.0–400 μM, respectively (Tibazarwa

Table 4. Genetically engineered microorganisms as biosensors.

Microorganism	Heavy metal	Lowest detectable conc.	Device	Reference
Staphylococcus aureus$^+$	Cd	10 nM	Luminometer	Tauriainen *et al.* 1998
	Pb	33 nM		
Escherichia coli$^+$	Cu	1.0 μM	Amperometer	Corbisier *et al.* 1999
	Cr	1.0 μM		
	Pb	0.5 μM		
Saccharomyces cerevisiae$^+$	Cu	0.1 mM	Amperometer	Lehmann *et al.* 2000
Ralstonia eutropha$^+$	Ni	1.0 mM	Luminometer	Tibazarwa *et al.* 2001
	Co	9.0 mM		

et al. 2001). Table 4 shows the recently developed genetically engineered microorganisms as biosensors for heavy metal. The prospective for future microbial biosensors, thus, is based on genetically engineered microorganism for toxicity and bioavailability testing (D'Souza 2001). Of related interest, GEM-based metal biosensors (BIOMET sensors) have been patented (US, 5, 786 162).

Conclusions

This review has presented an account of the different types of biosensors developed for the analysis of heavy metal ions. Protein-based biosensors which monitor enzyme inhibition or activation offer fast response times, however, specificity often is lacking in the inhibition assays. Non-enzymatic metal-binding regulatory proteins offer great promise as biosensors, as illustrated by potential sensitivities of detection as low as femto- and zepto-molar levels (Changela *et al*. 2003). The antibody-based immunosensors also are highly specific and sensitive. The whole-cell based biosensors offer certain advantages such as the microbial strains are cheaper than enzyme electrodes and the enzyme activity is enhanced in microbial cells owing to optimal environment, leading to greater stability, however, the microbial electrodes are more susceptible to interference and contamination. This has led to GEM-based biosensors using bacteria that are genetically engineered so that a quantifiable signal is produced when the bacteria are in contact with a specific metal ion. For this purpose, the promoter of the metal ion responding gene is ligated to the reporter gene. This approach, although adding to the cost of the process, is highly specific and likely will lead to rapid commercialization such as the BIOMET sensors, produced in Belgium.

References

Andres RT, Narayanaswamy R. 1995 Effect of the coupling reagent on the metal inhibition of immobilized urease in an optical biosensor. *Analyst* **120**, 1549–1554.

APHA-American Public Health Association. 1995 In: Eaton AE, Clesceri LS, Greenberg AE, eds., *Standard Methods for the Examination of Water and Wastewater*, 19th edn., Maryland: United Book Press Inc.

Aston WJ, Turner APF. 1984 In: Russel GE, ed., *Biotechnology and Genetic Engineering Reviews*, Vol. 1. Newcstle : Intercept : 89–120.

Barondeau DP, Kassmann CJ, Tainer JA, Getzoff ED. 2002 Structural chemistry of a green fluorescent protein Zn biosensor. *J Am Chem Soc* **124**, 3522–3524.

Blake DA. 1995 Quantitation of heavy metals by immunoassay. <http://es.epa.gov/ncer/final/grants/95/chemistrywater/blake.html>

Blake DA, Blake RC 2nd, Khosraviani M, Pavlov AR. 1998 Metal ion immunoassays. *Anal Chim Acta* **376**, 13–19.

Blake DA, Chakrabarti P, Khosraviani M, Hatcher FM, Westhoff CM, Goebel P, Wylie E, Blake RC. 1996 Metal binding properties of a monoclonal antibody directed towards metal–chelate complexes. *J Biol Chem* **271**, 27677–27685.

Blake DA, Jones RM, Blake RC 2nd, Pavlov AR, Darwish IA, Yu H. 2001 Antibody-based sensors for heavy metal ions. *Biosens Bioelectron* **16**(9–12), 799–809.

Bontidean I. 2002 Design, development and applications of highly sensitive protein based capacitive biosensors. Lund University Dissertation Abstracts.

Bontidean I, Ahlqvist J, Mulchandani A, Chen W, Bae W, Mehra RK, Mortari A, Csoregi E. 2003 Novel synthetic phytochelatin based capacitive biosensor for heavy metal detection. *Biosens Bioelectron* **18**(5–6), 547–553.

Breuil P, Benedelto DD, Poyet JP. 1998 Online analysis of copper and zinc in industrial effluents by UV-visible spectrometry. *Analysis* **26**(8), M63–M68.

Brodelius P, Vandamme EJ. 1987 In: Rehm HJ, Reed R, eds., *Biotechnology*, Vol. 7a. FR Germany: VCH Publishers; 407–464.

Brown NL, Lloyd JR, Jakeman KJ, Hobman JL, Bontidean I, Mattiasson B, Csöregi E. 1998 Heavy metal resistance genes and proteins in bacteria and their application. *Biochem Soc Trans* **26**, 662–665.

Burstein C, Adamovicz E, Boucherit K, Rabouielle C, Romette JL. 1986 Immobilized respiratory chain activities from *Escherichia coli* utilized to measure D-and L-lactate, succinate, L-malate, 3-glycerophosphate, pyruvate or NAD(P)H. *Appl Biochem Biotechnol* **12,** 1–15.

Changela A, Chen K, Xue Y, Holschen J, Outten CE, Halloran TV, Mondragon A. 2003 Molecular basis of metal ion selectivity and zeptomolar sensitivity by CueR. *Science* **301**, 1383–1386.

Corbisier P, Lelie D, Borremans B, Provoost A, Lorenzo V, Brown NL, Lloyd JR, Hobman JL, Csöregi E, Johansson G, Mattiasson B. 1999 Whole cell-and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples. *Anal Chim Acta* **387**, 235–244.

Corcoran CA, Rechnitz GA. 1985 Cell based biosensors. *Trends Biotechnol* **3**(4), 92–96.

Cowell DC, Dowman AA, Ashcroft T. 1995 The detection and identification of metal and organic pollutants in potable water using enzyme assays suitable for sensor development. *Biosens Bioelectron* **10**(6–7), 509–516.

D'Souza SF. 2001 Microbial biosensors. *Biosens Bioelectron* **16**, 337–353.

Davis J, Vaughan DH, Cardosi MF. 1995 Elements of biosensor construction. *Enz Microb Technol* **17**, 1030–1035.

Dennison MJ, Turner APF. 1995 Biosensors for environmental monitoring. *Biotechnol Adv* **13**, 1–12.

Fennouh S, Casimiri V, Meyer AG, Burstein C. 1998 Kinetic study of heavy metal salt effects on the activity of L-lactate

dehydrogenase in solution or immobilized on an oxygen electrode. *Biosens Bioelectron* **13**, 903–909.

Gayet JC, Haouz A, Meyer A, Burstein C. 1993 Detection of heavy metal salts with biosensors built with an oxygen electrode coupled to various immobilized oxidases and dehydrogenases. *Biosens Bioelectron* **8**, 177–183.

Gronow M. 1984 Biosensors. *Trends Biochem Sci* **9**, 336–340.

Guilbault GG. 1984 *Analytical Uses of Immobilized Enzymes*. New York: Marcel Dekker; 211–226.

Kennedy JF, Cabral JMS. 1987 In: Rehm HR, Reed G, eds., *Biotechnology*, Vol. 7a. FR Germany: VCH Publishers; 349–404.

Khosraviani M, Pavlov AR, Flowers GC, Blake DA. 1998 Detection of heavy metals by immunoassay: Optimization and validation of a rapid, portable assay for ionic cadmium. *Environ Sci Tech* **32**, 137–142.

Klaassen CD. 1996 In: Molinkoff PB, Ruddon RW, eds., *Goodman and Gilmans. The Pharmacological Basis of Therapeutics*; 1649–1650.

Krawczyk TK, Moszczyn'ska M, Trojanowicz M. 2000 Inhibitive determination of mercury and other metal ions by potentiometric urea biosensor. *Biosens Bioelectron* **15**, 681–691.

Lee S, Sode K, Nakanishi K, Marty JL, Tamiya E, Karube I. 1992 A novel microbial sensor using luminous bacteria. *Biosens Bioelectron* **7**, 273–277.

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.

Liu J, Lu Y. 2003 A colorimetric lead biosensor using DNAzyme-directed assembly of gold nanoparticles. *J Am Chem Soc* **125**, 6642–6643.

Luong JHT, Bouvrette P, Male KB. 1997 Developments and applications of biosensors in food analysis. *Trends Biotechnol* **15**, 369–377.

Luong JHT, Mulchandani A, Guilbault GG. 1988 Developments and applications of biosensors. *Trends Biotechnol* **6**, 310–316.

Maffia GM, Davis J. 2001 Bi-phasic collagen for the extraction of metals from contaminated waters. http://muse.widener.edu/soengr/srprojects2001.htm.

Mulchandani A, Bassi AS. 1995 Principles and applications of biosensors for bioprocess monitoring and control. *Cri Rev Biotechnol* **15**(2), 105–124.

Nam YJ, Kim N, Volotovsky V. 1997 Urease-based biosensor for mercuric ions determination. *Sensors Actuators* **B42**, 233–237.

Peavy HS, Rowe DR, Tchobanoglous G. 1988. *Environmental Engineering*. Singapore: McGraw Hill Inc.

Prasad S, Zhang X, Yang M, Ozkan CS, Ozkan M. 2004. Neurons as sensors: Individual and cascade chemical sensing. *Biosens Bioelectron* **19**(12), 1599–1610.

Preininger C, Wolfbeis O. 1996 Disposable cuvette test with integrated sensor layer for enzymatic determination of heavy metals. *Biosens Bioelectron* **11**, 981–990.

Riedel K, Kunze G, Konig A. 2002 Microbial sensors on a respiratory basis for wastewater monitoring. *Adv Biochem Engg Biotechnol* **75**, 81–118.

Rodriguez BB, Borbot JA, Tothill IE. 2004 Development of urease and glutamic dehydrogenase amperometric assay for heavy metals screening in polluted samples. *Biosens Bioelectron* **19**(1), 1157–1167.

Rogers KR, Lin JN. 1992 Biosensors for environmental monitoring. *Biosens Bioelectron* **7**, 317–321.

Satoh I. 1991 An apoenzyme thermistor microanlaysis for zinc (II) ions with use of an immobilized alkaline phosphatase reactor in a flow system. *Biosens Bioelectron* **6**(4), 375–379.

Scheller F, Schubert F. 1992 *Biosensors*. New York: Elsevier Science Publishers.

Shekhovtsova TN, Muginova SV, Bagirova NA. 1997 Determination of organomercury compounds using immobilized peroxidase. *Anal Chim Acta* **344**, 145–151.

Tauriainen S, Karp M, Chang W, Virta M. 1998 Luminiscent bacterial sensor for cadmium and lead. *Biosens Bioelectron* **13**, 931–938.

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**(1), 19–26.

Tiemann KJ, Jorge, Torresdey G. 1998 Use of X-ray absorption spectroscopy and esterification to investigate Cr(III) and Ni(II) ligands in alfalfa biomass. *Env Sci Technol* **33**(1), 150–154.

Togawa T, Tamura T, öberg PA. 1997 *Biomedical Transducers and Instruments*. New York: CRC Press.

Townsend AT, Miller KA, McLean S, Aldous S. 1998 The determination of copper, zinc, cadmium and lead in urine by high resolution ICP-MS. *J Anal Atomic Spectro* **13**(11), 1213–1219.

Turner APF, Karube I, Wilson GS, eds. 1987 *Biosensors Fundamentals and Applications*. Oxford, UK: Oxford University Press.

Verma N, Malaku ET. 2001 Studies on the development of disposable biosensor for monitoring malathion pesticide residues. In: *Biochemistry-Environment and Agriculture*. Ed. APS Mann; 265–269.

Verma N, Singh M. 2003 A disposable microbial based biosensor for quality control in milk. *Biosens Bioelectron* **18**(10), 1219–1224.

Yamasaki A, Cunha MA, Oliveira JA, Duarte AC, Gomes MT. 2004 Assessment of copper toxicity using an acoustic wave sensor. *Biosens Bioelectron* **19**(10), 1203–1208.

Yim HS, Kibbey CE, Ma SC, Kliza DM, Liu D, Park SB, Torre CE, Meyerhoff ME. 1993 Polymer membrane based ion-, gas-and bioselective potentiometric sensors. *Biosens Bioelectron* **8,** 1-38.

Zeng HH, Thompson RB, Maliwal BP, Fones GR, Mosffet JW, Fierke CA. 2003 Real-time determination of picomolar free Cu(II) in seawater using a fluorescence based fiber optic biosensor. *Anal Chem* **75**, 6807–6812.