Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytica Chimica Acta 568 (2006) 200–210
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Review

Microbial biosensors

Yu Lei [a,*], Wilfred Chen [b], Ashok Mulchandani [b,*]

[a] *Division of Chemical and Biomolecular Engineering and Centre of Biotechnology, Nanyang Technological University, Singapore 637722, Singapore*
[b] *Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521, USA*

Received 29 August 2005; received in revised form 17 November 2005; accepted 21 November 2005
Available online 18 January 2006

Abstract

A microbial biosensor is an analytical device that couples microorganisms with a transducer to enable rapid, accurate and sensitive detection of target analytes in fields as diverse as medicine, environmental monitoring, defense, food processing and safety. The earlier microbial biosensors used the respiratory and metabolic functions of the microorganisms to detect a substance that is either a substrate or an inhibitor of these processes. Recently, genetically engineered microorganisms based on fusing of the *lux*, *gfp* or *lacZ* gene reporters to an inducible gene promoter have been widely applied to assay toxicity and bioavailability. This paper reviews the recent trends in the development and application of microbial biosensors. Current advances and prospective future direction in developing microbial biosensor have also been discussed.

Keywords: Microbial biosensors; Amperometric; Potentiometric; Optical; Luminescence; Fluorescence

Contents

* Corresponding authors. Tel.: +65 67906712; fax: +65 67947553.
E-mail addresses: YLei@ntu.edu.sg (Y. Lei), adani@engr.ucr.edu (A. Mulchandani).

0003-2670/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.aca.2005.11.065

1. Introduction

A biosensor is an analytical device that combines a biological sensing element with a transducer to produce a signal proportional to the analyte concentration [1–18]. This signal can result from a change in protons concentration, release or uptake of gases, light emission, absorption and so forth, brought about by the metabolism of the target compound by the biological recognition element. The transducer converts this 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 [1–3].

Biomolecules such as enzymes, antibodies, receptors, organelles and microorganisms as well as animal and plant cells or tissues have been used as biological sensing elements. Among these, microorganisms offer advantages of ability to detect a wide range of chemical substances, amenability to genetic modification, and broad operating pH and temperature range, making them ideal as biological sensing materials [1–18]. Microorganisms have been integrated with a variety of transducers such as amperometric, potentiometric, calorimetric, conductimetric, colorimetric, luminescence and fluorescence to construct biosensor devices [1–8]. Several reviews papers and book chapters addressing microbial biosensor development have been published [1–20]. The intent of this review is to highlight the advances in the rapidly developing area of microbial biosensors with particular emphasis to the developments since 2000.

2. Advantages of using microorganisms as biosensing elements

Enzymes are the most widely used biological sensing element in the fabrication of biosensors [1–4]. Although purified enzymes have very high specificity for their substrates or inhibitors, their application in biosensors construction may be limited by the tedious, time-consuming and costly enzyme purification, requirement of multiple enzymes to generate the measurable product or need of cofactor/coenzyme. Microorganisms provide an ideal alternative to these bottle-necks [15]. The many enzymes and co-factors that co-exist in the cells give the cells the ability to consume and hence detect large number of chemicals; however, this can compromise the selectivity. They can be easily manipulated and adapted to consume and degrade new substrate under certain cultivating condition [21–23]. Additionally, the progress in molecular biology/recombinant DNA technologies has opened endless possibilities of tailoring the microorganisms to improve the activity of an existing enzyme or express foreign enzyme/protein in host cell [19,20]. All of these make microbes excellent biosensing elements.

3. Immobilization of microorganisms

The basis of a microbial biosensor is the close contact between microorganisms and the transducer. Thus, fabrication of a microbial biosensor requires immobilization on transducers with a close proximity. Since microbial biosensor response, operational stability and long-term use are, to some extent, a function of the immobilization strategy used, immobilization technology plays a very important role and the choice of immobilization technique is critical. Microorganisms can be immobilized on transducer or support matrices by chemical or physical methods [1–6].

3.1. Chemical methods

Chemical methods of microbe immobilization include covalent binding and cross-linking [1–6,24]. Covalent binding methods rely on the formation of a stable covalent bond between functional groups of the microorganisms' cell wall components such as amine, carboxylic or sulphydryl and the transducer such as amine, carboxylic, epoxy or tosyl. To achieve this goal, whole cells are exposed to harmful chemicals and harsh reaction condition, which may damage the cell membrane and decrease the biological activity. How to overcome this drawback is still a challenge for immobilization through covalent binding. To our knowledge, this method has therefore not been successful for immobilization of viable microbial cells [1–17,24].

Cross-linking involves bridging between functional groups on the outer membrane of the cells by multifunctional reagents such as glutaraldehyde and cyanuric chloride, to form a network. Because of the speed and simplicity, the method has found wide acceptance for immobilization of microorganisms. The cells may be cross-linked directly onto the transducer surface or on a removable support membrane, which can then be placed on the transducer [1–17,24]. The ability to replace the membrane with the immobilized cells is an advantage of the latter approach. While cross-linking has advantages over covalent binding, the cell viability and/or the cell membrane biomolecules can be affected by the cross-linking agents. Thus cross-linking is suitable in constructing microbial biosensors where cell viability is not important and only the intracellular enzymes are involved in the detection [8].

3.2. Physical methods

Adsorption and entrapment are the two widely used physical methods for microbial immobilization. Because these methods do not involve covalent bond formation with microbes and provide relatively small perturbation of microorganism native structure and function, these methods are preferred when viable cells are required [8,14–17,24].

Physical adsorption is the simplest method for microbe immobilization. Typically, a microbial suspension is incubated with the electrode or an immobilization matrix, such as alumina and glass bead [4,8,24], followed by rinsing with buffer to remove unadsorbed cells. The microbes are immobilized due to adsorptive interactions such as ionic, polar or hydrogen bonding and hydrophobic interaction. However, immobilization using adsorption alone generally leads to poor long-term stability because of desorption of microbes.

The immobilization of microorganisms by entrapment can be achieved by the either retention of the cells in close

proximity of the transducer surface using dialysis or filter membrane or in chemical/biological polymers/gels such as (alginate, carrageenan, agarose, chitosan, collagen, polyacrylamide, polyvinylachohol, poly(ethylene glycol), polyurethane, etc. [1–4,8,15]. A major disadvantage of entrapment immobilization is the additional diffusion resistance offered by the entrapment material, which will result in lower sensitivity and detection limit.

Microbial biosensor can be classified based on the transducers into electrochemical, optical and others.

4. Electrochemical microbial biosensor

There are three types of electrochemical microbial biosensors: amperometric, potentiometric, and conductometric [1–6].

4.1. Amperometric microbial biosensor

Amperometric microbial biosensor operates at fixed potential with respect to a reference electrode and involves the detection of the current generated by the oxidation or reduction of species at the surface of the electrode. Table 1 summarizes a few of the amperometric biosensors reported in the literature.

Amperometric microbial biosensors have been widely developed for the determination of biochemical oxygen demand (BOD) for the measurement of biodegradable organic pollutants in aqueous samples [25]. The conventional standard method for the determination of BOD measures the microorganisms' oxygen consumption/respiration over a period of 5 days [26,27] and is reported as BOD_5 [25,28]. While BOD_5 is a good indicator of the concentration of organic pollutants in water, it is extremely slow and hence not suitable for process control [29,30]. To address this limitation, several BOD biosensors based on amperometric oxygen electrode transducer modified with microorganisms degrading/metabolizing organic pollutants have been reported [25–30]. The microbial strains used as biological sensing element include *Torulopsis candida* [31], *Trichosporon cutaneum* [32,33], *Pseudomonas putida* [34], *Klebsiella oxytoca* AS1 [35], *Bacillus subtilis* [36,37], *Arxula adeninivorans* LS3 [38–41], *Serratia marcescens* LSY4 [42], *Pseudomonas* sp. [43], *P. fluorescens* [44,45], *P. putida* SG10 [46], *Thermophilic bacteria* [47], *Hansennula anomala* [48] and yeast [29]. Because any given strain provides a narrow substrate spectrum, single-strain-BOD-biosensor has limitations in analyzing complex samples. This bottleneck can be alleviated by employing a mixture of two or more microorganisms to broaden the substrate and hence analyte spectrum with a stable performance [49–57].

As the most extensively investigated microbial biosensor, the first commercial BOD biosensor was produced by Nisshin Denki (Electric) in 1983. Since then, several more BOD biosensors have been commercialized by DKK Corporation, Japan; Autoteam FmbH, Germany; Prufgeratewerk Medingen GmbH, Germany; Dr. Lange GmbH, Germany; STIP Isco GmbH, Germany; Kelma, Belgium; LAR Analytik and Umweltmesstechnik GmbH, Germany; Bioscience, Inc., USA; USFilter, USA [8,25].

While most of the research and development in BOD biosensors has focused in identifying different microorganisms that can determine BOD of a specific waste, research efforts have also been directed at improving the amperometric transducer itself. For example, a miniaturized oxygen electrode based on thick-film screen-printing was recently developed to replace the bulky Clark dissolved oxygen electrode transducer. The widely used thick-film screen-printing technique was used to print the platinum-working electrode, Ag/AgCl reference electrode and platinum auxiliary electrode of the amperometric oxygen electrode on an inert substrate. The oxygen electrode was then modified with *A. adeninivorans* LS3 by entrapment in poly(carbamoyl) sulfonate (PCS) gel and successfully applied for rapid (∼100 s) and stable (up to 2 months) BOD determination [26]. Similarly, to extend the dynamic range of the BOD sensor, which in the case of dissolved oxygen electrode is limited by the solubility of oxygen in the sample, a ferricyanide-mediated microbial biosensor using a novel yeast strain for BOD measurement was developed [58]. Recently, an amperometric transducer array featuring four individually addressable platinum electrodes was constructed and modified with two microbial strains with different substrate spectra for the measurement of BOD and poly cyclic aromatic hydrocarbons (PAH) simultaneously [28].

Besides BOD biosensor, amperometric microbial biosensors have also been applied for measurement of several other chemicals. Because of its importance in fermentation industry and clinical toxicology [8,59], microbial biosensors for ethanol has garnered the second most research attention after BOD. Different microorganisms metabolizing ethanol such as *Trichosporon brassicae* [60], *Acetobacter aceti* [61], *Candida vini* [62], *Gluconobacter suboxydans* [63], *C. tropicalis* [64], *Aspergillus niger* [65], *Saccharomyces ellipsoideus* [66], *G. oxydans* [59] and *Pichia methanolica* [59] have been immobilized on oxygen electrode to fabricate ethanol biosensors. While these biosensors posses good sensitivity and stability, they usually have poor selectivity. Thus, there is a great interest to develop selective microbial ethanol biosensor. An improved selectivity for ethanol determination in presence of glucose was achieved by replacing oxygen with ferricyanide as the electron acceptor mediator for *G. oxydans* immobilized on a glassy carbon electrode by cellulose acetate membrane which also restricted the availability of glucose to the cells by size exclusion [67,68].

Sugars are important ingredients of different media and sensors for determination of sugars are therefore highly desired. Microbial biosensors for sugars have ranged from the simple modification of Clark and microfabricated oxygen electrode with *S. cerevisiae* and *E. coli* K12 mutants, respectively, to modification of graphite electrode with *G. oxydans* in conjunction with hexacyanoferrate (III) as a mediator [69–71].

Phenol and substituted phenols have received considerable attention in waste analysis program due to their high toxicity to mammals, humans and plants. A variety of amperometric microbial biosensors [72–78] have been reported for these EPA Priority chemicals. *p*-Nitrophenol (PNP) degrading bacterial *Arthrobacter* JS 443 and *Moraxella* sp. isolated from PNP contaminated sites in the U.S. have been immobilized on oxygen and carbon paste electrodes using polycarbonate membrane

Table 1
Amperometric microbial biosensors

Target	Microorganism	Limit of detection	References
BOD	*A. adeninivorans* LS3	1.24 mg/l	[26]
BOD	*C. parapsilosis*	1 mg/l	[28]
BOD	yeast	1 mg/l	[29]
BOD	*T. cutaneum* and *B. subtilis*	0.5 mg/l	[30]
BOD	*T. candida*	7–75 ppm	[31]
BOD	*T. cutaneum*	0–32 mg/l	[32]
BOD	*T. cutaneum*	10–70 mg/l	[33]
BOD	*P. putida*	0.5 mg/l	[34]
BOD	*K. oxytoca* AS1	<44 mg/l	[35]
BOD	*B. subtilis* (heat killed)	10–80 mg/l	[36]
BOD	*B. subtilis*	2–22 mg/l	[37]
BOD	*A. adeninivorans* LS3	8–550 mg/l	[38]
BOD	*A. adeninivorans* LS3	2 mg/l	[39]
BOD	*A. adeninivorans* LS3	–	[40]
BOD	*A. adeninivorans* LS3	2.61 mg/l	[41]
BOD	*Serratia marcescens* LSY4	0–44 ppm	[42]
BOD	*Pseudomonas* sp.	1–40 mg/l	[43]
BOD	*P. fluorescens*	15–200 mg O/l	[44]
BOD	*P. fluorescens*	15–260 mg/l	[45]
BOD	*P. putida* SG10	1 mg/l	[46]
BOD	*T. bacteria*	<10 mg/l	[47]
BOD	*BODSEED*	5–45 mg/l	[49]
BOD	*Rhodococcus erythropolis* DSM Nr. 772 and *Issatchenkia orientalis* DSM Nr. 3433	–	[50]
BOD	*B. subtilis* and *B. licheniformis 7B*	10–70 mg/l	[51]
BOD	*B. subtilis* and *B. licheniformis 7B*	0–80 mg/l	[52]
BOD	*B. subtilis* and *B. licheniformis 7B*	0–70 mg/l	[53]
BOD	*Microbial consortium*	5.0 mg BOD_5/l	[55,56]
BOD	*M. consortium*	1 mg/l	[57]
BOD	*Yeast SPT1* and *SPT2*	2 mg/l	[58]
Ethanol	*G. oxydans* or *P. methanolica*	0.05 mM	[59]
Ethanol	*A. aceti* (IFO 3284)	<0.2 mM	[61]
Ethanol	*C. vini*	0.02–0.2 mM	[62]
Ethanol	*G. suboxydans*	0–25 mg/l	[63]
Ethanol	*C. tropicalis*	0.5–7.5 mM	[64]
Ethanol	*A. niger*	1–32 ppm	[65]
Ethanol	*S. ellipsoideus*	69 μM	[66]
Ethanol	*G. oxydans*	0.85 μM	[67,68]
Total sugars	*G. oxydans*	1.1–2.2 g/l	[69]
Sucrose	*S. cerevisiae*	6–100 mM	[70]
Mono-and /disaccharides	*E. coli* K12	0–4 mM for disaccharides 0–2.5 mM for monosaccharide	[71]
p-Nitrophenol	*A.* JS 443	5 nM	[72]
p-Nitrophenol	*A.* JS 443	0.2 μM	[73]
p-Nitrophenol	*M.* sp.	0.1 μM	[74]
p-Nitrophenol	*M.* sp.	20 nM	[75]
2,4-Dinitrophenol	*R. erthropolis*	2–20 μM	[76]
Phenolic compounds	*P. putida*	0.5–6 μM, 0.3–2.5 μM and 0.02–0.2 μM	[77]
Phenolic compounds	*P. putida*	0.1–1.0 μM and 0.05–1.0 μM	[78]
Organophosph ates	Recombinant *Moraxella*	0.2 μM paraoxon and 1 μM methyl parathion	[80]
Organophosph ates	Recombinant *P. putida* JS 444	55 ppb of paraoxon, 53 ppb of methyl parathion, and 58 ppb of parathion	[81]
Cyanide	*S. cerevisiae*	0.15 μM	[82]
Cyanide	*T. ferrooxidans*	0.5 μM	[83]
Cyanide	*P. fluorescents* NCIMB 11764	0.05–1 mg/l	[86]
Cyanide	*S. cerevisiae* IFO 0377	0–15 μM	[87]
Cyanide	*S. cerevisiae*	0.3–150 μM	[88]
Anionic surfactants	*Pseudomonas* and *Archromobacter*	1 μlM	[89]
Non-ionic surfactants	*Comamonas testosterone* TI	0.25 mg/l	[90]
Hydrogen peroxide	*A. peroxydans*	0.1–9.5 μM	[91]
Acetic acid	*F. solani*	2–70 ppm (v/v)	[92]
Microbiologic-ally influenced corrosion	*P.* sp.	0–0.7 mM sulphuric acid	[93]
Cu^{2+}	Recombinant *S. cerevisiae*	0.5–2 mM	[95]
Cadmium	Recombinant *E. coli*	25 nM	[96]

[73,74] and Nafion [72], respectively, and by directly mixing in the carbon paste [75] to fabricate biosensor for PNP. Other microbial biosensors for phenols include *Rhodococcus erthropolis* modified Clark oxygen electrode for 2,4-dinitrophenol (2,4-DNP) [76] and *P. putida* DSM 50026, a well-known phenol degrading microorganisms, modified thick-film and screen-printed graphite electrodes for phenols [77,78].

Neurotoxic organophosphate (OP) compounds have found wide applications as pesticides and insecticides in agriculture and as chemical warfare agents in military practice [8,79–81]. Amperometric biosensors based on genetically engineered *Moraxella* sp. and *P. putida* with surface-expressed organophosphorus hydrolase (OPH) have been developed for sensitive, selective and cost-effective detection of OPs. These biosensors relied on the amperometric detection of PNP generated from hydrolysis of OP compounds by surface-displayed OPH or oxygen consumed and electrochemically active intermediates formed during the further mineralization of the PNP by the cells [80,81].

The inhibition of bacterial respiration and hence the decrease of oxygen consumption rate, has been utilized to fabricate cyanide biosensor [82,83]. Whole-cell biosensors consisting of dissolved oxygen electrode modified with *Nitrosomonas europaea*, *Thiobacillus ferrooxidans*, *Saccharomyces cerevisiae* and *Pseudomonas fluorescens* were reported for batch and continuous cyanide monitoring [83–88].

Other amperometric microbial biosensors based on monitoring of cell respiration include biosensor for surfactants, representing a widespread group of organic pollutants, using surfactant-degrading bacteria [89,90], hydrogen peroxide by coupling immobilized living *Acetobacter peroxydans* [91] and for acetic acid using *Fusarium solani* [92].

Over the last two decades, the microbiologically influenced corrosion (MIC) of metallic materials has received great attention. A stable, reproducible and specific microbial biosensor was developed for monitoring MIC of metallic materials in industrial systems based on *Pseudomonas* sp. isolated from corroded metal surface and immobilized on acetylcellulose membrane at oxygen electrode. A linear relationship between the biosensor response and the concentration of sulfuric acid (the most corrosive inorganic acid involved in microbial corrosion) was established. The biosensor response time was 5 min and was dependent on many parameters such as pH, temperature, corrosive environment and immobilized cell loading [93]. The same group also used *Acetobacter* sp. to develop amperometric microbial biosensor for monitoring microbiologically influenced corrosion caused by fungal species [94].

Another application of amperometric microbial biosensors is the detection of heavy metal ions for environmental control. A microbial biosensor to detect Cu^{2+} by an amperometric method has been developed using recombinant *S. cerevisiae* containing plasmids with Cu^{2+}-inducible promoter fused to the *lacZ* gene. In the presence of Cu^{2+}, the recombinant strains are able to utilize lactose as a carbon source and lead to the oxygen consumption change, which can be detected by using oxygen electrode [95]. A novel promoter-based electrochemical biosensor for on-line and in situ monitoring of gene expression in response to cadmium has also been described [96]. A cadmium-responsive promoter from *E. coli* was fused to a promoterless *lacZ* gene, and then the β-galactosidase activity was monitored using screen-printed electrode in the presence of cadmium [96]. This whole-cell biosensor could detect nanomolar concentrations of cadmium on-line or in-site within minutes.

4.2. Potentiometric microbial biosensor

Conventional potentiometric microbial biosensors consist of an ion-selective electrode (pH, ammonium, chloride and so on) or a gas-sensing electrode (p_{CO_2} and p_{NH_3}) coated with an immobilized microbe layer. Microbe consuming analyte generates a change in potential resulting from ion accumulation or depletion. Potentiometric transducers measure the difference between a working electrode and a reference electrode, and the signal is correlated to the concentration of analyte [2,3,16]. Due to a logarithmic relationship between the potential generated and analyte concentration, a wide detection range is possible. However, this method requires a very stable reference electrode, which may be a limitation of these transducers. A few examples of biosensors based on potentiometric transducers are summarized in Table 2.

The simplest potentiometric microbial biosensor is based on the modification ion selective electrode. Several microbial biosensors based on modification of glass pH electrode with genetically engineered *E. coli* expressing organophosphorus hydrolase intracellularly and on the outer surface of cells and

Table 2
Potentiometric microbial biosensors

Target	Microorganism	Transducer type	Limit of detection	References
Organophosphates	*Flavobacteium* sp.	pH electrode	0.025–0.4 mM	[97]
Organophosphates	Recombinant *E coli*	pH electrode	2 μM	[98]
Organophosphates	Recombinant *E coli*	pH electrode	3 μM	[99]
Penicillin	Recombinant *E coli*	Flat pH electrode	5–30 mM	[100]
Penicillin	Recombinant *E coli*	pH electrode	1–16 mM	[101]
Tryptophan	*E coli* WP2	LAPS	0–12 μM	[102]
Urea	*Bacillus* sp.	NH_4^+ ion selective electrode	0.55–550 μM	[103]
Trichloroethylene	*P. aeruginosa* JI104	Chloride ion selective electrode	0.03–2 mg/l	[104]
Trichloroethylene	*P. aeruginosa* JI104	Chloride ion selective electrode	0.1–4 mg/l	[105]
Ethanol	*S. ellipsoideus*	Oxygen	0.02–50 mM	[106]
Sucrose	*S. cerevisiae*	Oxygen	3.2 μM	[107]

wild-type OP degrading bacteria *Flavobacteium* sp. have been reported [16,97–99]. The principle of detection is based on the detection of the protons released by OPH catalyzed hydrolysis of OP and correlating to the concentration of OPs. Similarly, recombinant *E. coli* harboring the plasmids encoding for β-lactamase [100] and penicillinase [101] synthesis immobilized on pH electrode using gluten and acetylcellulose membranes entrapment, respectively, were developed for monitoring penicillin [100,101]. A new type of solid state silicon-based light addressable potentiometric sensor for monitoring hydrogen ion was integrated to the auxotrophic bacteria *E. coli* WP2 (requiring tryptophan for its growth) to fabricate a potentiometric microbial assay for tryptophan [102].

While pH electrodes are the most widely applied ion selective electrode for microbial biosensors, other ion selective electrodes have also been utilized. For example, an ammonium ion selective electrode was coupled with urease-yielding *Bacillus* sp. isolated from soil to develop a disposable microbial biosensor for monitoring the presence of urea in milk [103]. Similarly, a chloride ion selective electrode was modified with TCE degrading bacterium *Pseudomonas aeruginosa* JI104 for TCE monitoring in batch and continuous modes in wastewaters [104,105].

A potentiometric oxygen electrode with immobilized *S. ellipsoideus* was also successfully used to produce a microbial biosensor for the determination of ethanol with an extended response range [106]. Based on the same format, sucrose biosensor based on an immobilized *S. cerevisiae* was also described [107].

4.3. Conductimetric biosensor

Many microbe-catalyzed reactions involve a change in ionic species. Associated with this change is a net change in the conductivity of the reaction solution. Even though the detection of solution conductance is non-specific, conductance measurements are extremely sensitive [1–3].

Recently, a single-use conductivity and microbial sensor were developed to investigate the effect of both species and concentration/osmolarity of anions on the metabolic activity of *E. coli*. This hybrid sensing system combines physico-chemical and biological sensing and greatly increases the ease with which comparative data could be assimilated [108].

4.4. Microbial fuel cell type biosensor

Microbial fuel cells (MFCs) have been studied as a BOD sensor for a long time. Since Karube et al. reported a BOD sensor based on MFC using the hydrogen produced by *Clostridium butyricum* immobilized on the electrode in 1977 [109], a variety of MFC BOD sensors with use of electron-mediator have been developed [110–112]. Even though the addition of mediators in these biosensors can enhance the electron transfer, these biosensors have poor stability because of the toxicity of mediators. Recently, mediator-less microbial fuel cells have been exploited to fabricate novel BOD sensors for continuous and real-time monitoring [113,114]. Furthermore, Kim et al. reported that the performance of a microbial fuel cell as BOD sensor was improved using respiratory inhibitors [115].

5. Optical microbial biosensor

The modulation in optical properties such as UV–vis absorption, bio- and chemi-luminescence, reflectance and fluorescence brought by the interaction of the biocatalyst with the target analyte is the basis for optical microbial biosensors [1–5]. Optical-based biosensors offer advantages of compactness, flexibility, resistance to electrical noise, and a small probe size. Some representative bioluminescence and fluorescence based microbial biosensors are listed in Table 3.

5.1. Bioluminescence biosensor

Bioluminescence is associated with the emission of light by living microorganisms and it plays a very important role in real-time process monitoring. The bacterial luminescence *lux* gene has been widely applied as a reporter either in an inducible or constitutive manner. In the inducible manner, the reporter *lux* gene is fused to a promoter regulated by the concentration of a compound of interest. As a result, the concentration of the compound can be quantitatively analyzed by detecting the bioluminescence intensity [19,20]. In the constitutive manner, the reporter gene is fused to promoters that are continuously expressed as long as the organism is alive and metabolically active [19]. This kind of reporter is good for evaluating the total toxicity of contaminant. Both types of reporters have been shown to be useful for biosensor development.

Heavy metal-mediated toxicity in the environment is dependant on bioavailable metal concentrations. Bioluminescent microbial biosensors have been extensively investigated to monitor bioavailable metal. *Ralstonia eutropha* AE2515 was constructed by transcriptionally fusing *cnr*YXH regulatory genes to the bioluminescent *lux*CDABE report system to fabricate a whole cell biosensor for the detection of bioavailable concentration of Ni^{2+} and Co^{2+} in soil [116]. Several optical biosensors consisting of bacteria that contain gene fusion between the regulatory region of the *mer* operon (*mer*R) and *lux*CDABE have been developed to quantitatively response to Hg^{2+}. The *mer* promoter is activated when Hg^{2+} binds to *Mer*R, then result the transcription of the *lux* reporter gene and subsequent light emission [117–122]. Bioavailable copper in soil is also monitored by using engineered *P. fluorescens* through mutagenesis of *P. fluorescens* containing copper-induced gene and *Tn5::lux*AB promoter probe transposon [123].

In order to monitor nutrients in an aquatic ecosystem, a biosensor for monitoring phosphorus bioavailablity to *Cyanobacteria* (*Synechococcus* PCC 7942) was developed [124]. The reporter strain *Synechococcus* harbors the gene coding the reporter protein luciferase under the control of an inducible alkaline phosphatase promoter, which can be induced under phosphorous limitation and shows improvement to conventional phosphorus detection methods [124]. Bioluminescent microbial biosensors using the inducible reporter gene have also been developed for the measurement of bioavailable naph-

Table 3
Bioluminescence and fluorescence microbial biosensors

Target	Microorganism	Transducer type	Limit of detection	References
Ni^{2+} and Co^{2+}	*Ralstonia eutropha* AE2515	Luminescence	0.1 μM Ni^{2+}, 9 μM Co^{2+}	[116]
Urinary mercury (II)	*E. coli* MC1061 harboring *mer-lux* plasmid pTOO11	Luminescence	1.67×10^{-13} M	[117]
Hg^{2+}	*E. coli* HMS174 harboring *mer-lux* plasmid pRB27 or pRB28	Luminescence	0.2 ng/g	[118]
Bioavailable mercury	*E. coli* HMS174 harboring *mer-lux* plasmid pRB27 or pRB28	Luminescence	–	[119]
Bioavailable mercury	*E. coli* HMS174 harboring *mer-lux* plasmid pRB27	Luminescence	10 pM	[121]
Bioavailable mercury	*E. coli* HMS174 harboring *mer-lux* plasmid pRB28, pOS14orpOS15	Luminescence	nM level	[122]
Bioavailable copper	*P. fluorescens* DF57 with a Tn5*::*luxAB promoter probe transposon	Luminescence	0.3 ppm	[123]
Bioavailable phosphorus	*Synechococcus* PCC 7942 reporter strain	Luminescence	0.3 μM	[124]
Bioavailable naphthalene	*P. putida* carrying NAH7 plasmid and a chromosomally inserted gene fusion between the *sal* promoter and the *luxAB* genes	Luminescence	50–500 nM	[125]
Tributyltin	Bioluminescent recombinant *E. colir::luxAB* strain	Luminescence	0.02 μM in synthetic glucose medium and 1.5 μM in LB medium	[126]
Halogenated organic acids	Recombinant *E. coli* containing DL-2-haloacid dehalogenase encoding gene and *luxCDABE* genes	Luminescence	>100 mg/l	[127]
Water pollutants/toxicity	*E.* co//HB101 pUCD607 containing *luxCDABE* cassette	Luminescence	On-line test EC_{50} values ranged from 0.09 to 21 mg/l for different pillutants	[128]
Pollutants/toxicity	*E. coli* bearing *fab A'::lux* fusions	Luminescence	EC50 values (mg/l) dependent on tested pollutants	[129]
Pollution- induced stress	*P. fluorescens* pUCD607	Luminescence	∼2 ppm	[130]
Toxicity of chlorophenol	*P. fluorescens* 10586r pUCD607	Luminescence	EC_{50} values (mg/l) dependent on tested pollutants	[131]
Toxicity of waster water treatment plant treating phenolics-containing waster	*P.fischeri* and *P. putida BS566::luxCDABE*	Luminescence	EC_{50} values (g/l) ranged from 0.034 to 0.638	[132]
Genotoxicants	*E. coli* DPD1718 containing *recA'::lux* fusion	Luminescence	100 ppb mitomycin	[133]
UV	Recombinant *E. coli* containing *recA'::lux* fusion	Luminescence	1.2 J/cm^2 UV dose	[134]
UV	*P. aeruginosa* FRD1 carrying plasmid pMOE15 with *recA::luxCDABE*	Luminescence	–	[135]
Bioavailable iron	Recombinant *Pseudomonas syringae* carrying *gfp* gene	Fluorescence	$\sim 10^{-7}$ M	[136]
Arsenite	*E. coli* DH5α (pPR-arsR-ABS, expressing *egfp*)	Fluorescence	–	[137]
Galactosides	*Sinorhizobium meliloh* containing a *gfp* gene fused to the *melA* promoter	Fluorescence	–	[138]
Bioavailable toluene and related compounds	*P. fluorescens* A506 (pTolLHB)and E. *cloacae* JL1157 (pTolLHB)	Fluorescence	0.02 μM toluene	[139]
N-Acyl homoserine lactones in soil	*E. coli* MC4100 harboring pAHL-GFP	Fluorescence	–	[140]
Water availability	*E. coli, Pantoea agglomerans* and *Pseudomonas syringae* harbouring the fusion of proU promoter and *gfp* gene	Fluorescence	–	[141]
Cell population	*E. coll* JM-109 pQE60-EGFP	Fluorescence	–	[142]
BOD	Oxygen-sensitive fluorescent material and sea water microorganisms	Fluorescence	4 mg/l	[143]
BOD	*P. putida* and optical fiber sensor from ASR Co. Ltd.	Fluorescence	0.5 mg/l	[144]

thalene [125], tributyltin [126] and halogenated organic acids [127].

The environmental problems caused by industrial and agricultural pollution have increased the demand for the development of pollutant and toxicity detection methods. The fusion of reporter genes to promoters that are induced when cell are stressed by toxic chemicals are one promising approach that has been used to fabricate biosensor for such application. Recombinant *E. coli* bearing *fabA'::lux* fusion and plasmid pUCD607 containing the full *luxCDABE* cassette have been constructed as biosensors for water pollutant detection [128,129]. The online pollutant and toxicity test, using bioluminescence-based biosensors, was proved to be sensitive and reliable. *Lux*-marked rhizobacterium *P. fluorescens* has been developed to evaluate the pollution-induced stress, which influences rhizobacterium carbon flow based on the fact that bioluminescence output of biosensor is directly correlated with metabolic activity and reports on carbon flow in root exudates [130]. Furthermore, *lux*-marked whole cell biosensors for evaluation of interactive toxicity of chlorophenol [131] and toxicity assessment of a wastewater treatment plant treating phenolics-containing waste [132] have been reported, respectively. These biosensors responded to tested pollutants fast and enable a rapid toxicity test possible.

Genotoxicants is a class of hazards, which can cause DNA damage. An optical-fiber bioluminescent microbial sensor to detect the DNA damage hazard-mitomycin C by the induction of a selected promoter and the subsequent production of bioluminescent light through a recombinant *lux* reporter was reported. Bioluminescence production was shown to be dose-dependent [133]. *E. coli* containing plasmid-borne fusion of the *recA* promoter-operator region to the *Vibrio fischeri lux* genes has also been reported for genotoxicant detection. When the recombinant *E. coli* strains are challenged with DNA damage hazards, they increase their luminescence [134]. Furthermore, this study was expanded by investigating and demonstrating the luminescence response of these strains to ultraviolet radiation, which can cause DNA damage [134]. Another *lux*-based *Psedumonoas aeruginosa* biosensor was fabricated to quantify bacteria exposure to UV radiation in biofilm [135].

5.2. Fluorescence biosensor

Fluorescence spectroscopy has been widely applied in analytical chemistry. It is a sensitive technique that can detect very low concentrations of analyte because of the instrumental principles involved. At low analyte concentrations, fluorescence emission intensity is directly proportional to the concentration. Fluorescent materials and green fluorescent protein have been extensively used in the construction of fluorescent biosensor [1–5].

5.2.1. Green fluorescence protein-based biosensor

Like bioluminescent reporter *lux* gene, *gfp* gene coding for the green fluorescent protein (GFP) has also been widely applied as reporters and fused to the host gene that allows reporter activity to be examined in individual cells. Because GFP is very stable and not known to be produced by microorganism indigenous to terrestrial habits, it provides great advantage and flexibility when evaluating reporter activity. The primary disadvantage of GFP as a reporter protein is the delay between protein production and protein fluorescence.

The GFP-based microbial biosensor has been shown to be useful in assessing heterogeneity of iron bioavailability on plant [136]. In this sensor, ferric iron availability to cells was assessed by quantifying the fluorescence intensity of cells containing a plasmid-borne transcriptional fusion between an ion-regulated promoter and *gfp* [136]. Recently, Wells et al. developed an ultrasensitive biosensor for arsenite by using laser-induced fluorescence confocal spectroscopy to measure arsenite-stimulated enhanced green fluorescent protein synthesis of genetically engineered *E. coli* bioreporter cell, which has an inherent single-molecule detection capability [137]. A recombinant soil bacterium *Sinorhizobium meliloti* has been constructed by fusing the *gfp* gene to the *melA* promoter, which is induced on exposure to galactose and galactosides. Using this fusion strain, a biosensor was developed to determine the concentration of galactosides [138]. Similarly, *gfp* reporter gene has also been used to develop biosensors for various applications, such as detecting bioavailable toluene and related compounds [139] and *N*-acyl homoserine lactones in soil [140], measuring water availability in a microbial habitat [141], monitoring cell populations [142] and so on. With the development of DNA recombinant technologies and our understanding to microbes, this type of biosensor will become an increasingly more powerful technique.

5.2.2. O_2-sensitive fluorescent material-based biosensor

Besides green fluorescent protein, other fluorescent materials have also been used in the construction of microbial biosensor. Recently, fiber-optical microbial sensors for determination of BOD were reported [143,144]. The biosensors consisted of either a layer of oxygen-sensitive fluorescent materials that are made up of seawater microorganisms immobilized in poly(vinyl alcohol) sol–gel matrix and an oxygen fluorescence quenching indicator with linear range of 4–200 mg/l [143], or an immobilized *P. putida* membrane attached to an optical fiber sensor for dissolved oxygen from ASR Co. Ltd. with detection limit of 0.5 mg/l [144].

5.3. Colorimetric biosensor

A sensitive biosensor based on color changes in the toxin-sensitive colored living cells of fish was reported [145]. In the presence of toxins produced by microbial pathogens, the cells undergo visible color change and the color changes in a dose-dependant manner. 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.

We reported a simple fiber-optic based microbial sensor to detect organophosphates based on the absorbance of PNP formed from the hydrolysis of organophosphates by the genetically engineered *E. coli* expressing organophosphorus hydrolase

on the cell surface [146]. This biosensor can be easily extended to other organophosphates such as coumaphos through the monitoring of its hydrolysis product coumarin.

A colorimetric whole cell bioassay for the detection of common environmental pollutants benzene, toluene, ethyl benzene and xylene (BTEX), found at underground fuel storage tanks, using recombinant *E. coli* expressing toluene dioxygenase and toluene dihydrodiol dehydrogenase was reported. The bioassay was based on the enzyme catalyzed conversion of the BTEX components to their respective catechols followed by the reaction with hydrogen peroxide in presence of horseradish peroxidase to colrimetric products that can be monitored at 420 nm [147].

6. Other types of microbial biosensors

Besides electrochemical, optical and colorimetric microbial biosensors, there are few other types of biosensors reported recently.

6.1. Sensors based on baroxymeter for the detection of pressure change

As a new application, baroxymeter has been developed as a portable wastewater direct toxicity assessment device based on manometric bacterial respirometry. Respirometry was measured as the pressure drop in the headspace of a close vessel due to oxygen uptake by the microorganism in contact with sample. This microbial pressure sensor showed good reproducibility and comparable responses with other reported methods [148].

6.2. Sensors based on infrared analyzer for the detection of the microbial respiration product CO_2

A new method was reported for monitoring inhibitory effects in wastewater treatment plants based on continuous measurement of the microbial respiration product CO_2. Activated sludge microbes are used as the biological elements and their respiratory activity is inhibited by the presence of toxic compounds, resulting in a decrease in CO_2 concentration which was analyzed by using a CO_2 infrared analyzer [149]. Based on the measurement of CO_2 concentration in the off gas produced during degradation of carbon compound by microbial respiration activities, a microbial biosensor was developed to monitor the extent of organic pollution in wastewater both off-line in a laboratory and online in a wastewater treatment plant [150].

7. Future trends

Since Clark and Lyons developed the first biosensor, the field of biosensors has greatly expanded. Microorganisms, due to their low cost, long lifetime and wide range of suitable pH and temperature, have been widely employed as the biosensing element in the construction of biosensors [2]. Even though a variety of microbial biosensors have been developed for environmental, food, military and biomedical application, when compared to enzyme biosensors the development of highly satisfactory microbial biosensor is still hampered because they suffer from long response time, low sensitivity and poor selectivity.

With a better understanding of the genetic information of microbes and the development of improved recombinant DNA technologies, different enzymes and proteins have been expressed on the cell surface through surface expression anchors. In this format, the microbes can serve as an enzymes' support matrix. The surface expressed enzymes or proteins can directly react with substrates without the entry of substrates into the microbes. Through this way, faster response and highly sensitive microbial biosensors can be developed [80,81,98,146]. The same surface expression system can also be applied to produce other pollutant-resistant strains for biosensor fabrication.

Because non-specific cellular response to substrates and intermediates of microbial catabolism can limit the selectivity of microbial biosensors, the ability to design, select and screen for microorganisms with specific activity for certain chemical compounds will play an important role in the development of high selectivity microbial biosensors. To achieve this goal, it is necessary to combine the classical knowledge in microbiology with the rapidly expanding methodologies in genetic engineering in order to control or create microbe's metabolic pathway (on/off) according to our purpose.

Another trend in microbial biosensors is to develop biosensors for the application in extreme conditions, such as highly acidic, alkaline, saline, extreme temperature and organic solvent environment because more and more detections will involve such unfriendly conditions. Since normal microbes can not survive in such harsh environment, the selection of microorganism, which survive under these extreme conditions while maintaining high enzymes activities will become more and more important in future development of microbial biosensor. These requirements are particularly important for the growing biosensor industry because of the great need for low cost, sensitive, selective and fast response biosensor in the market [151]. We believe, with current advances in microbial biosensor and progress in modern biotechnology, microbial biosensors will have a promising and bright future.

Acknowledgements

We greatly appreciate the support of U.S. EPA and USDA for supporting studies on microbial biosensors.

References

[1] A.P.F. Turner, I. Karube, G.S. Wilson (Eds.), Biosensors: Fundamentals and Applications, Mir Publishers, Moscow, 1992.
[2] A. Mulchandani, K.R. Rogers (Eds.), Enzyme and Micriobial Biosensors: Techniques and Protocols, Humana Press, Totowa, NJ, 1998.
[3] M.C. Tran, Biosensors, Chapman and Hall and Masson, Paris, 1993.
[4] S.R. Mikkelsen, E. Cortón, Bioanalytical Chemistry, John Wiley and Sons, New Jersey, 2004.
[5] L.J. Blum, P.R. Coulet (Eds.), Biosensor Principles and Applications, Marcel Dekker, New York, 1991.
[6] D. Nikolelis, U. Krull, J. Wang, M. Mascini (Eds.), Biosensors for Direct Monitoring of Environmental Pollutants in Field, Kluwer Academic, London, 1998.

[7] G. Ramsay (Ed.), Commercial Biosensors: Applications to Clinical, Bioprocess and Environmental Samples, Wiley, Chichester, UK, 1998.
[8] S.F. D'Souza, Biosens. Bioelectron. 16 (1997) 337.
[9] I. Karube, J. Biotechnol. 15 (1990) 255.
[10] I. Karube, T. Matsunaga, S. Mitsuda, S. Suzuki, Microbial electrode BOD sensors, Biotechnol. Bioeng. 19 (1977) 1535.
[11] Y.I. Korpan, A.V. Elskaya, Biochem. (Moscow) 60 (1995) 1517.
[12] D.G. Margineanu, H. Vais, I. Ardelean, J. Biotechnol. 3 (1985) 1.
[13] Y. Nomura, I. Karube, in: R. Williaert, G. Baron, L. De Backer (Eds.), Biosensor Using Immobilized Living Cells in Immobilized Living Cell Systems, Wiley, Chichester, UK, 1996, p. 345.
[14] K. Riedel, in: A. Mulchandani, K.R. Rogers (Eds.), Enzyme and Microbial Biosensors: Techniques and Protocols, Humana Press, Totowa, NJ, 1998, p. 199.
[15] Y. Arikawa, K. Ikebukuro, I. Karube, in: A. Mulchandani, K.R. Rogers (Eds.), Enzyme and Microbial Biosensors: Techniques and Protocols, Humana Press, Totowa, NJ, 1998, p. 225.
[16] A.L. Simonian, E.I. Rainina, J.R. Wild, in: A. Mulchandani, K.R. Rogers (Eds.), Enzyme and Microbial Biosensors: Techniques and Protocols, Humana Press, Totowa, NJ, 1998, p. 237.
[17] U. Matrubutham, G.S. Sayler, in: A. Mulchandani, K.R. Rogers (Eds.), Enzyme and Microbial Biosensors: Techniques and Protocols, Humana Press, Totowa, NJ, 1998, p. 249.
[18] H. Nakamura, I. Karube, Anal. Bioanal. Chem. 377 (2003) 446.
[19] C. Rensing, R.M. Maier, Ecotoxicol. Environ. Safety 56 (2003) 140.
[20] S. Belkin, Curr. Opin. Microbiol. 6 (2003) 206.
[21] R.K. Jain, J.H. Dreisbach, J.C. Spain, Appl. Environ. Microbiol. 60 (1994) 3030.
[22] K.T. Leung, O. Tresse, D. Errampalli, H. Lee, J.T. Trevors, FEMS Microbiol. Lett. 155 (1997) 107.
[23] J.C. Spain, S.F. Nishino, Appl. Environ. Microbiol. 53 (1987) 1010.
[24] S.F. D'Souza, Appl. Biochem. Biotechnol. 96 (2001) 225.
[25] J. Liu, B. Mattiasson, Water Res. 36 (2002) 3786.
[26] C. Chan, M. Lehmann, K. Chan, P. Chan, C. Chan, B. Gruendig, G. Kunze, R. Renneberg, Biosens. Bioelectron. 15 (2000) 343.
[27] APHA, Standard Methods for the Examination of Waters and Wastewater, 16th ed., American Public Hearth Association, Washington, DC, 1986, p. 525.
[28] A. König, T. Reul, C. Harmeling, F. Spener, M. Knoll, C. Zaborosch, Anal. Chem. 72 (2000) 2022.
[29] D. Chen, Y. Cao, B. Liu, J. Kong, Anal. Bioanal. Chem. 372 (2002) 737.
[30] J. Jia, M. Tang, X. Chen, L. Qi, S. Dong, Biosens. Bioelectron. 18 (2003) 1023.
[31] S. Sangeetha, G. Sugandhi, M. Murugesan, V.M. Madhav, S. Berchmans, R. Rajasekar, S. Rajasekar, D. Jeyakumar, G.P. Rao, Electroanalysis 8 (1996) 698.
[32] Z. Yang, S. Sasaki, I. Karube, H. Suzuki, Anal. Chim. Acta 357 (1997) 41.
[33] J.L. Marty, D. Olive, Y. Asano, Environ. Technol. 18 (1997) 333.
[34] G.-J. Chee, Y. Nomura, I. Karube, Anal. Chim. Acta. 379 (1999) 185.
[35] A. Ohki, K. Shinohara, O. Ito, K. Naka, S. Maeda, T. Sato, H. Akano, N. Kato, Y. Kawamura, J. Int. Environ. Anal. Chem. 56 (1994) 261.
[36] T.C. Tan, Z.-R. Qian, Sens. Actuators B 40 (1997) 65.
[37] K. Riedel, R. Renneberg, M. Kuehn, F. Scheller, Appl. Microbiol. Biotechnol. 28 (1988) 316.
[38] K. Riedel, M. Lehmann, K. Tag, R. Renneberg, G. Kunze, Anal. Lett. 31 (1998) 1.
[39] C. Chan, M. Lehmann, K. Tag, M. Lung, G. Kunze, K. Riedel, B. Gruendig, R. Renneberg, Biosens. Bioelectron. 14 (1999) 131.
[40] K. Tag, A.W.K. Kwong, M. Lehmann, C. Chan, R. Renneberg, K. Riedel, G. Kunze, J. Chem. Technol. Biotechnol. 75 (2000) 1080.
[41] K. Tag, M. Lehmann, C. Chan, R. Renneberg, K. Riedel, G. Kunze, Sens. Actuators B 67 (2000) 142.
[42] M. Kim, H. Kwon, Biosens. Bioelectron. 14 (1999) 1.
[43] Y.-R. Li, J. Chu, Appl. Biochem. Biotechnol. 28–29 (1991) 855.
[44] N. Yoshida, K. Yano, T. Morita, S.J. McNiven, H. Nakanura, I. Karube, Analyst 125 (2000) 2280.
[45] N. Yoshida, J. Hoashi, T. Morita, S.J. McNiven, H. Nakanura, I. Karube, J. Biotechnol. 88 (2001) 269.
[46] G.-J. Chee, Y. Nomura, K. Ikebukuro, I. Karube, Biosens. Bioelectron. 21 (2005) 67.
[47] I. Karube, K. Yokoyama, K. Sode, E. Tamiya, Anal. Lett. 22 (1989) 791.
[48] J. Kulys, K. Kadziauskiene, Biotechnol. Bioeng. 22 (1980) 221.
[49] T.C. Tan, C. Wu, Sens. Acutators B 54 (1999) 252.
[50] S. Heim, I. Schnieder, D. Binz, A. Vogel, U. Bilitewski, Biosens. Bioelectron. 14 (1999) 187.
[51] T.C. Tan, F. Li, K.G. Neoh, Y.K. Lee, Sens. Actuators B 8 (1992) 167.
[52] T.C. Tan, F. Li, K.G. Neoh, Sens. Actuators B 10 (1993) 137.
[53] F. Li, T.C. Tan, Y.K. Lee, Biosens. Bioelectron. 9 (1994) 197.
[54] K. Riedel, Vom. Wasser. 81 (1993) 243.
[55] J. Liu, G. Olsson, B. Mattiasson, Biosens. Bioelectron. 20 (2004) 562.
[56] J. Liu, G. Olsson, B. Mattiasson, Biosens. Bioelectron. 20 (2004) 571.
[57] S. Rastogi, A. Kumar, N.K. Mehra, S.D. Makhijani, A. Manoharan, V. Gangal, R. Kumar, Biosens. Bioelectron. 18 (2003) 23.
[58] S.P. Trosok, B.T. Driscoll, J.H.T. Luong, Appl. Microbiol. Biotechnol. 56 (2001) 550.
[59] A.N. Reshetilov, J.A. Trotsenko, N.O. Morozova, P.V. Iliasov, V.V. Ashin, Process Biochem. 36 (2001) 1015.
[60] I. Karube, in: A.P.F. Turner, I. Karube, G.S. Wilson (Eds.), Biosensors: Fundamentals and Applications, Mir Publishers, Moscow, 1992, p. 20.
[61] T. Ikeda, K. Kato, M. Maeda, H. Tatsumi, K. Kano, K. Matsushita, J. Electroanal. Chem. 430 (1997) 197.
[62] M. Mascini, A. Memoli, F. Olana, Enzyme Microbiol. Technol. 11 (1989) 297.
[63] Y. Kitagawa, M. Ameyama, K. Nakashima, E. Tamiya, I. Karube, Analyst 112 (1987) 1747.
[64] E. Akyilmaz, E. Dinckaya, Biosens. Bioelectron. 20 (2005) 1263.
[65] S. Subrahmanyam, K. Shanmugam, T.V. Subramanian, M. Murugesan, V.M. Madhav, D. Jeyskumar, Electroanalysis 13 (2001) 944.
[66] L. Rotariu, C. Bala, Anal. Lett. 36 (2003) 2459.
[67] J. Tkac, I. Vostiar, P. Gemeiner, E. Sturdik, Bioelectrochemistry 56 (2002) 127.
[68] J. Tkac, I. Vostiar, L. Gorton, P. Gemeiner, E. Sturdik, Biosens. Bioelectron. 18 (2003) 1125.
[69] J. Tkac, P. Gemeiner, J. Svitel, T. Benikovsky, E. Sturdik, V. Vala, L. Petrus, E. Hrabarova, Anal. Chim. Acta 420 (2000) 1.
[70] L. Rotariu, C. Bala, V. Magearu, Revue Roumaine de Chimie 45 (2000) 21.
[71] M. Held, W. Schuhmann, K. Jahreis, H.L. Schmidt, Biosens. Bioelectron. 17 (2002) 1089.
[72] Y. Lei, P. Mulchandani, W. Chen, J. Wang, A. Mulchandani, Eletroanalysis 16 (2004) 2030.
[73] Y. Lei, P. Mulchandani, W. Chen, J. Wang, A. Mulchandani, Eletroanalysis 15 (2003) 1160.
[74] P. Mulchandani, Y. Lei, W. Chen, J. Wang, A. Mulchandani, Anal. Chim. Acta 470 (2002) 79.
[75] P. Mulchandani, M.H. Carlos, Y. Lei, W. Chen, A. Mulchandani, Anal. Biosens. Bioelectron. 21 (2005) 523.
[76] E.V. Emelyanova, A.N. Reshetilov, Process Biochem. 37 (2002) 683.
[77] S. Timur, N. Pazarlioglu, R. Pilloton, A. Telefoncu, Talanta 61 (2003) 87.
[78] S. Timur, L.D. Seta, N. Pazarlioglu, R. Pilloton, A. Telefoncu, Process Biochem. 39 (2004) 1325.
[79] USDA, Agricultural Satistics, US Government Printing Office, Washington, DC, 1992, p. 395.
[80] P. Mulchandani, W. Chen, A. Mulchandani, J. Wang, L. Chen, Biosens. Bioelectron. 16 (2001) 433.
[81] Y. Lei, P. Mulchandani, W. Chen, A. Mulchandani, J. Agric. Food Chem. 53 (2005) 524.
[82] K. Ikebukuro, K.M. Honda, K. Nakanishi, Y. Nomura, Y. Masuda, K. Yokoyama, Y. Yamauchi, I. Karube, Electroanalysis 8 (1996) 876.
[83] M. Okochi, K. Mima, M. Miyata, Y. Shinozaki, S. Haraguchi, M. Fujisawa, M. Kaneko, T. Masukata, T. Matsunaga, Biotechnol. Bioeng. 87 (2004) 905.

[84] E. Nakamura, H. Tanaka, Y. Tanaka, H. Hoshikawa, T. Kato (1990) Japanese Patent No. Hei2-190763.
[85] T. Shinohara, Y. Tanaka (1989) Japanese Patent No. Show64-63850.
[86] J.I. Lee, I. Karube, Electroanalysis 8 (1996) 1117.
[87] K. Ikebukuro, A. Miyata, S.J. Cho, Y. Nomura, S.M. Chang, Y. Yamauchi, Y. Hasebe, S. Uchiyama, I. Karube, J. Biotechnol. 48 (1996) 73.
[88] K. Nakanishi, K. Ikebukuro, I. Karube, Appl. Biochem. Biotechnol. 60 (1996) 97.
[89] L.A. Taranova, I. Semenchuk, T. Manolov, P. Iliasov, A. Reshetilov, Biosens. Bioelectron. 17 (2002) 635.
[90] L.A. Taranova, A.P. Fesay, G.V. Ivashchenko, A.N. Reshetilov, M. Winther-Nielsen, J. Emneus, Appl. Biochem. Microbiol. 40 (2004) 404.
[91] S. Rajasekar, R. Rajasekar, K.C. Narasimhan, Bull. Electrochem. 16 (2000) 25.
[92] S. Subrahmanyam, N. Kodandapani, K. Shanmugam, K. Moovarkumuthalvan, D. Jeyakumar, T.V. Subramanian, Electroanalysis 13 (2001) 1275.
[93] R.S. Dubey, S.N. Upadhyay, Biosens. Bioelectron. 16 (2001) 995.
[94] R.S. Dubey, S.N. Upadhyay, Indian J. Chem. Technol. 10 (2003) 607.
[95] M. Lehmann, K. Riedel, K. Adler, G. Kunze, Biosens. Bioelectron. 15 (2000) 211.
[96] I. Biran, R. Babai, K. Levcov, J. Rishpon, E.Z. Ron, Environ. Microbiol. 2 (2000) 285.
[97] S. Gaberlein, F. Spener, C. Zaborosch, Appl. Microbiol. Biotechnol. 54 (2000) 652.
[98] A. Mulchandani, P. Mulchandani, I. Kaneva, W. Chen, Anal. Chem. 70 (1998) 4140.
[99] A. Mulchandani, S. Chauhan, I. Kaneva, P. Mulchandani, W. Chen, Electroanalysis 10 (1998) 733.
[100] E. Galindo, D. Bautisa, J.L. Garcia, R. Quintero, Enzyme Microb. Technol. 12 (1990) 642.
[101] H.P. Chao, W.C. Lee, Biotechnol. Appl. Biochem. 32 (2000) 9.
[102] A. Seki, K. Kawakubo, M. Iga, S. Nomura, Sens. Actuators B 94 (2003) 253.
[103] N. Verma, M. Singh, Biosens. Bioelectron. 18 (2003) 1219.
[104] T.S. Han, S. Sasaki, K. Yano, K. Ikebukuro, A. Kitayama, T. Nagamune, I. Karube, Talanta 57 (2002) 271.
[105] T.S. Han, Y.C. Kim, S. Sasaki, K. Yano, K. Ikebukuro, A. Kitayama, T. Nagamune, I. Karube, Anal. Chim. Acta 431 (2001) 225.
[106] L. Rotariu, C. Bala, V. Magearu, Anal. Chim. Acta 513 (2004) 119.
[107] L. Rotariu, C. Bala, V. Magearu, Anal. Chim. Acta 458 (2002) 215.
[108] R. Bhatia, J.W. Dilleen, A.L. Atkinson, D.M. Rawson, Biosens. Bioelectron. 18 (2003) 667.
[109] I. Karube, T. Matsunga, S. Mitsuda, S. Suzuki, Biotechnol. Bioeng. 19 (1977) 1535.
[110] T. Matsunaga, I. Karube, S. Suzuki, Eur. J. Appl. Microbiol. Biotechnol. 10 (1980) 235.
[111] J.L. Stirling, H.P. Bennetto, G.M. Delaney, J.R. Mason, S.D. Roller, K. Tanaka, C.F. Thurston, Biochem. Soc. Trans. 11 (1983) 451.
[112] C.F. Thurston, H.P. Bennetto, G.M. Delaney, J.R. Mason, S.D. Roller, J.L. Stirling, J. Gen. Microbiol. 131 (1985) 1393.
[113] I.S. Chang, J.K. Jang, G.C. Gil, M. Kim, H.J. Kim, B.W. Cho, B.H. Kim, Biosens. Bioelectron. 19 (2004) 607.
[114] B.H. Kim, I.S. Chang, G.C. Gil, H.S. Park, H.J. Kim, Biotechnol. Lett. 25 (2003) 541.
[115] I.S. Chang, H. Moon, J.K. Jang, B.H. Kim, Biosens. Bioelectron. 20 (2005) 1856.
[116] C. Tibazarwa, P. Corbisier, M. Mench, A. Bossus, P. Solda, M. Mergeay, L. Wyns, D. van derLelie, Environ. Pollut. 113 (2001) 19.
[117] A. Roda, P. Pasini, M. Mirasoli, M. Guardigli, C. Russo, M. Musiani, M. Baraldini, Anal. Lett. 34 (2001) 29.
[118] L.D. Rasmussen, S.J. Sorensen, R.R. Turner, T. Barkay, Soil Biol. Biochem. 32 (2000) 639.
[119] T. Barkay, M. Gillman, R.R. Turner, Appl. Environ. Microbiol. 63 (1997) 4267.
[120] T. Barkay, R.R. Turner, L.D. Rasmussen, C.A. Kelly, J.W.M. Rudd, Luminescence facilitated detection of bioavailable mercury in natural waters, in: R.A. LaRossa (Ed.), Bioluminescence Methods and Protocols, Methods in Molecular Biology, vol. 102, Humanae Press, Totowa, NJ, 1998.
[121] L.D. Rasmussen, R.R. Turner, T. Barkay, Appl. Environ. Microbiol. 63 (1997) 3291.
[122] O. Selifonova, R. Burlage, T. Barkay, Appl. Environ. Microbiol. 59 (1993) 3083.
[123] A. Tom-Petersen, C. Hosbond, O. Nybroe, FEMS Microbiol. Ecol. 38 (2001) 59.
[124] P.P.Y. Schreiter, O. Gillor, A. Post, S. Belkin, R.D. Schmid, T.T. Bachmann, Biosens. Bioelectron. 16 (2001) 811.
[125] C. Werlen, M.C.M. Jaspers, J.R. van de Meer, Appl. Environ. Microbiol. 70 (2004) 43.
[126] G. Thouand, H. Horry, M.J. Durand, P. Picart, L. Bendriaa, P. Daniel, M.S. DuBow, Appl. Microbiol. Biotechnol. 62 (2003) 218.
[127] M. Tauber, R. Rosen, S. Belkin, Talanta 55 (2001) 959.
[128] A.M. Horsburgh, D.P. Mardlin, N.L. Turner, R. Henkler, N. Strachan, L.A. Glover, G.I. Paton, K. Killham, Biosens. Bioelectron. 17 (2002) 495.
[129] O. Bechor, D.R. Smulski, T.K. van Dyk, R.A. LaRossa, S. Belkin, J. Biotechnol. 94 (2002) 125.
[130] F. Porteous, K. Killham, A. Meharg, Chemosphere 41 (2000) 1549.
[131] T. Tiensing, N. Strachan, G.I. Paton, J. Environ. Monitor. 4 (2002) 482.
[132] J.C. Philp, S. Balmand, E. Hajto, M.J. Bailey, S. Wiles, A.S. Whitely, A.K. Lilley, J. Hajto, S.A. Dunbar, Anal. Chim. Acta 487 (2003) 61.
[133] B. Polyak, E. Bassls, A. Novodvorets, S. Belkin, R.S. Marks, Water Sci. Technol. 42 (2000) 305.
[134] R. Rosen, Y. Davidov, R.A. Larossa, S. Belkin, Appl. Biochem. Biotechnol. 89 (2000) 151.
[135] M.O. Elasri, R.V. Miller, Appl. Environ. Microbiol. 65 (1999) 2025.
[136] D.C. Joyner, S.E. Lindow, Microbiology 146 (2000) 2435.
[137] M. Wells, M. Gosch, R. Rigler, H. Harms, T. Lasser, J.R. van de Meer, Anal. Chem. 77 (2005) 2683.
[138] R.M. Bringhurst, Z.G. Cardon, D.J. Gage, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 4540.
[139] N.C. Casavant, D. Thompson, G.A. Beattle, G.J. Phillips, L.J. Halverson, Environ. Microbiol. 5 (2003) 238.
[140] M. Burmolle, L.H. Hansen, G. Oregaard, S.J. Sorensen, Microbiol. Ecol. 45 (2003) 226.
[141] C.A. Axtell, G.A. Beattie, Appl. Environ. Microbiol. 68 (2002) 4604.
[142] D.O. Fesenko, T.V. Nasedkina, D.V. Prokopenko, A.D. Mirzabekov, Biosens. Bioelectron. 20 (2005) 1860.
[143] Y.J. Dai, L. Lin, P.W. Li, X. Chen, X.R. Wang, K.Y. Wong, Int. J. Environ. Anal. Chem. 84 (2004) 607.
[144] G.-J. Chee, Y. Nomura, K. Ikebukuro, I. Karube, Biosens. Bioelectron. 15 (2000) 371.
[145] K.P. Thomas, F.W. Chaplen, G. Jovanovic, W. Kolodziej, J.E. Trempy, C. Willard, J.A. Liburdy, D.V. Pence, B.K. Paul, Proc. SPIE 5321 (2004) 265.
[146] A. Mulchandani, I. Kaneva, W. Chen, Anal. Chem. 70 (1998) 5042.
[147] Z. Xu, A. Mulchandani, W. Chen, Biotechnol. Prog. 19 (2003) 1812.
[148] A. Tzoris, V. Fernandez-Perez, E.A.H. Hall, Sens. Actuators B 105 (2005) 39.
[149] A. Aivasidis, P. Melidis, D. Georgiou, Bioproc. Biosyst. Eng. 25 (2002) 29.
[150] E. Vaiopoulou, P. Melidis, E. Kampragou, A. Aivasidis, Biosens. Bioelectron. 21 (2005) 365.
[151] E.C. Alocilja, S.M. Radke, Biosens. Bioelectron. 18 (2003) 841.