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Review

Microbial biosensors: A review

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This review is dedicated to the memory of Mr. Chonggang Lei whose standards and lifelong achievements serve to inspire Dr. Yu Lei.

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ABSTRACT

A microbial biosensor is an analytical device which integrates microorganism(s) with a physical transducer to generate a measurable signal proportional to the concentration of analytes. In recent years, a large number of microbial biosensors have been developed for environmental, food, and biomedical applications. Starting with the discussion of various sensing techniques commonly used in microbial biosensing, this review article concentrates on the summarization of the recent progress in the fabrication and application of microbial biosensors based on amperometry, potentiometry, conductometry, voltammetry, microbial fuel cell, fluorescence, bioluminescence, and colorimetry, respectively. Prospective strategies for the design of future microbial biosensors will also be discussed.

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1. Introduction

A biosensor is an analytical device which integrates a biological recognition element with a physical transducer to generate a measurable signal proportional to the concentration of the analytes (Belkin, 2003; Cunningham, 1998; Eggins, 2002; Lei et al., 2006a; Sadana, 2001; Wilson and Gifford, 2005; Wilson, 2005). In the general scheme of a biosensor, the biological recognition element responds to the target compound and the transducer converts the biological response to a detectable signal, which can be measured electrochemically, optically, acoustically, mechanically, calorimetrically, or electronically, and then correlated with the analyte concentration (Byfield and Abuknesha, 1994; Cunningham, 1998; Eggins, 2002; Wilson, 2005). Since Clark and Lyon developed the first biosensor for glucose detection in 1962, biosensors have been intensively studied and extensively utilized in various applications, ranging from public health and environmental monitoring to homeland security and food safety (Amine et al., 2006; Kissinger, 2005; Lazcka et al., 2007; Patolsky et al., 2006; Wilson and Gifford, 2005).

Various biological recognition elements, including cofactors, enzymes, antibodies, microorganisms, organelles, tissues, and cells from higher organisms, have been used in the fabrication of biosensors (Lei et al., 2006a). Among these biological elements, enzymes are the most widely used recognition element due to their unique specificity and sensitivity (D'Souza, 2001b). However, the purification of enzyme is costly and time-consuming. In addition, the *in vitro* operating environment could result in a decrease of the enzyme activity (Byfield and Abuknesha, 1994). Microbes (e.g., algae, bacteria, and yeast) offer an alternative in the fabrication of biosensors because they can be massively produced through cell-culturing. Also, compared to other cells from higher organisms such as plants, animals, and human beings, microbial cells are easier to be manipulated and have better viability and stability *in vitro* (Byfield and Abuknesha, 1994), which can greatly simplify the fabrication process and enhance the performance of biosensors. Microbes are analogous to a "factory" consisting of numerous enzymes and cofactors/coenzymes, endowing themselves with the ability to respond to a number of chemicals, which can be used as the signal for sensing purposes. Even though metabolisms of the microorganisms are non-specific, highly selective microbial biosensors can be potentially achieved by blocking the undesired or inducing the desired metabolic pathway and by adapting the microorganisms to an appropriate substrate of interest (target) through selective cultivation conditions. Furthermore, recent development in molecular biology offers a novel method to construct genetically engineered microorganisms (GEMs), thus providing a new direction to manipulate the selectivity and sensitivity of microbial biosensors at the DNA level (Belkin, 2003; Paul et al., 2005; Urgun-Demirtas et al., 2006).

In order to effectively transform the biochemical response into a physical signal, the microbial cells, which serve as the recognition element in the biosensor, must be associated intimately with the transducer. Therefore, immobilizing microorganisms on transducers plays an important role in the fabrication of microbial biosensors (D'Souza, 2001b). Traditional methods for the immobilization of microorganisms include adsorption, encapsulation, entrapment, covalent binding, and cross-linking (Belkin, 2003; Bjerketorp et al., 2006; Cunningham, 1998; D'Souza, 2001a, 2001b; Ding et al., 2008; Eggins, 2002; Lei et al., 2006a; Xu et al., 2006).

Besides these methods, many novel immobilization strategies have been explored in recent years in order to improve the analytical performance and storage stability of the microbial biosensor. Song et al. developed a hybrid entrapment-encapsulation technique which combined advantages of the respective method and showed higher activity of immobilized cells compared to the single immobilization approach (Song et al., 2005). Yu et al. described an all-aqueous sol-gel method for the entrapment of *Moraxella* spp. cells where all immobilization procedures were carried out under mild conditions. Specifically, in contrast to the traditional alkoxide sol-gel technique, the application of sodium silicate as a precursor avoided the production of alcohol which could be detrimental to microbes, resulting in improved cell activity and viability (Yu et al., 2005). Flemming et al. fabricated a microfluidic channel for the gentle packing and immobilization of yeast cells. This method could achieve a higher density of active cells and less diffusion resistance inherent in the traditional entrapment technique (Flemming et al., 2006). Furthermore, a silicate network derived from the sol-gel technique provided a promising platform for microbe immobilization since it was possible to control the pore size of the entrapment materials, and thus increased the mass transport of analytes (Bjerketorp et al., 2006; Coradin et al., 2006). The immobilization of microorganisms in conducting polymer (CP) for microbial biosensor has also attracted much attention due to the unique electrochemical properties of CPs (Ahuja et al., 2007; Malhotra et al., 2006).

In past decades, several review papers have been published to address the development of microbial biosensors (Aller and Castro, 2006; Belkin, 2003; Borisov and Wolfbeis, 2008; Byfield and Abuknesha, 1994; D'Souza, 2001b; Ding et al., 2008; Du et al., 2007; Galluzzi and Karp, 2006; Karube, 1990; Lei et al., 2006a; Nakamura and Karube, 2003; Rogers, 2006; Yagi, 2007). This review article tends to discuss the recent achievements in the fabrication and application of microbial biosensors since 2005. Future trends of microbial biosensors will also be discussed.

2. Sensing techniques

Upon the addition of analytes to the immobilized microbes, the interaction between microbes and target compounds can be detected by a number of analytical techniques using suitable transducers. The measured signal can be correlated with the analyte concentration. Among various sensing techniques, electrochemical and optical techniques are most widely used in the development of microbial biosensors.

2.1. Electrochemical techniques

Electrochemical approaches are widely used in the development of microbial biosensors. According to the detection principle, electrochemical techniques can be divided into amperometry, potentiometry, conductometry, voltammetry, and microbial fuel cell (MFC).

Amperometry is operated at a given applied potential between the working electrode and the reference electrode and the current signal is recorded and correlated with the concentration of target compounds. In the amperometric detection, the current signal is generated due to the reduction or oxidation of an electroactive metabolic product or intermediate on the surface of a working electrode (Ding et al., 2008). Due to the intrinsic sensitivity of current measurement (down to picoampere), ultra-sensitive amperometric microbial biosensors can be easily realized.

Conductometry is a technique depending on the conductivity change in the solution due to the production or consumption of ionic species, for example, by the metabolic activity of the microorganisms (Lei et al., 2006a; Shulga et al., 1994). The measurement of conductance is extremely fast and sensitive under sophisticated modern analytical techniques, making conductometric microbial biosensors very attractive. It is also worth noting that such biosensors are suitable for miniaturization since it requires no reference electrode in the system (Shulga et al., 1994). However, all charge

carriers could result in the change of conductivity, thus the selectivity of conductometric biosensors is relatively poor (Mikkelsen and Rechnitz, 1989).

Potentiometry involves the measurement of the potential difference between the working electrode and the reference electrode and the potential signal exhibits concentration-dependent behavior. The transducer employed in the potentiometric technique is usually a gas-sensing electrode or an ion-selective electrode (Bobacka et al., 2008; Lei et al., 2006a). The sensitivity and selectivity of potentiometric biosensor are outstanding due to the species-selective working electrode used in the system. However, a highly stable and accurate reference electrode is always required and challenging to maintain, which may potentially limit the application of potentiometry in microbial biosensors.

Voltammetry is the most versatile technique in electrochemical analysis. Both the current and the potential are measured and recorded. The position of peak current is related to the specific chemical and the peak current density is proportional to the concentration of the corresponding species. A remarkable advantage of voltammetry is the low noise which can endow the biosensor with higher sensitivity. In addition, voltammetry is able to detect multiple compounds, which have different peak potentials, in a single electrochemical experiment (or scan), thus offering the simultaneous detection of multiple analytes. Furthermore, an effective pre-concentration step (electrochemical stripping analysis) makes the voltammetric technique one of the most sensitive electroanalytical methods (Wang, 1985).

MFC converts chemical energy into electrical energy by means of the metabolic activity of microorganisms (Rabaey and Verstraete, 2005). Since the consumption of target compounds by microbes or the inhibition of the metabolic pathway(s) by toxic compounds can potentially alter the production of electricity, MFC can be applied as a microbial biosensor for *in situ* analysis and for monitoring target chemicals.

2.2. Optical techniques

Optics is another commonly used technique in microbial biosensors. Optical detection is usually based on the measurement of luminescent, fluorescent, colorimetric, or other optical signals produced by the interaction of microorganisms with the analytes and correlates the observed optical signal with the concentration of target compounds. Genetically engineered microorganisms have been widely applied in optical whole-cell biosensors. A reporter gene is fused with an inducible gene. In the presence of a target analyte, the inducible gene is activated and consequently activates ("turn on") or represses ("turn off") the expression of a reporter gene which is responsible for the production of measurable optical signal (Borisov and Wolfbeis, 2008; Galluzzi and Karp, 2006). Optical sensing techniques are especially attractive in high throughput screening since they enable biosensors to monitor multiple analytes simultaneously (Brogan and Walt, 2005).

The *fluorescent sensing technique* is based on the measurement of fluorescence intensity which is proportional to the concentration of the target analyte. Fluorescence can be detected at a longer wavelength after the excitation of the fluorescent substance at a shorter wavelength. Fluorescent biosensors have been widely applied in analytical chemistry due to their easy construction using standard molecular biology techniques (Ibraheem and Campbell, 2010). Fluorescence-based microbial biosensors can be divided into *in vivo* and *in vitro* type. For the *in vivo* type fluorescent microbial biosensor, the microorganisms are able to produce the fluorescent substance (e.g., green fluorescent protein) without the addition of an exogenous fluorescent element. For the *in vitro* type, the metabolic activity of microorganisms changes the environment surrounding them, resulting in the change of light emission due to the exogenous fluorescent element.

The *bioluminescent sensing technique* used in microbial biosensors relies on detecting the change of luminescence emitted by living microorganisms which responds to the target analyte in a dose-dependent manner. The *lux* gene, encoding luciferase in microorganisms, is the most popular reporter gene in bioluminescent microbial biosensors (Borisov and Wolfbeis, 2008). Luciferase catalyzes the oxidation of flavin mononucleotide ($FMNH_2$) and the long-chain fatty aldehyde (RCHO) by oxygen with the emission of blue-green light (Galluzzi and Karp, 2006; Meighen, 1991). The strength of the bioluminescence is a measurement of enzyme activity instead of the quantification of protein, which is fundamental to fluorescent biosensors. Therefore, there is no surprise that bioluminescence provides a faster and more sensitive detection approach than fluorescence (Belkin, 2003). Another advantage of bioluminescent microbial biosensors is that, when the whole *lux* operon (*luxCDABE*) is employed as the reporter system, no exogenous substrate is required since all reactants ($FMNH_2$, RCHO, and oxygen), cofactors, and luciferase are produced *in vivo* (Borisov and Wolfbeis, 2008; Greer and Szalay, 2002). In addition, the expression of *lux* gene can be controlled in either a constitutive or an inducible manner (Galluzzi and Karp, 2006; Lei et al., 2006a).

The *colorimetric sensing technique* in microbial biosensors involves the conversion of a chromogen substrate into a colorful compound by the metabolic activity of the microbial sensing element (Azevedo et al., 2005). The colored product can be distinguished by the naked eye or a spectrophotometer. Because of its simple and inexpensive measurement setup, colorimetric technique has been widely applied in the fabrication of cost-effective microbial biosensors.

3. Recent progress in microbial biosensors

Microbial biosensor is a rapidly developing research area and there are numerous relevant publications since 2005. The intent of this article is to review the recent progress in the development of microbial biosensors. Based on the sensing technique, recent reported microbial biosensors can be classified into two major groups: electrochemical microbial biosensors and optical microbial biosensors.

3.1. Electrochemical microbial biosensors

3.1.1. Amperometric microbial biosensors

Amperometry is the most widely used technique in electrochemical microbial biosensors. Table 1 summarizes recently reported amperometric microbial biosensors in the literature.

Amperometric microbial biosensors have been extensively exploited for environmental applications (D'Souza, 2001b; Rogers, 2006; Yagi, 2007). Synthetic organophosphorus (OP) compounds, which cause severe nerve and muscular disorders (Singh, 2009), have been used worldwide as pesticides, petroleum additives, and chemical warfare agents (D'Souza, 2001b; Lei et al., 2006a). Several genes encoding the OP-degrading enzyme have been identified and, with the benefit of molecular biology technology, applied in whole-cell biosensors as the recognition element (Lei et al., 2006a; Singh, 2009). For example, genetically engineered *p*-nitrophenol (PNP)-degrader *Pseudomonas putida* JS444 and *Moraxella* sp., displaying organophosphorus hydrolase (OPH) activity, were constructed to selectively and sensitively detect paraoxon, methyl parathion, parathion, fenitrothion, and ethyl *p*-nitrophenol thiobenzenephosphonate (EPN) (Lei et al., 2005a, 2006b, 2007; Lei et al., 2005b; Mulchandani et al., 2006). OPH hydrolyzed these OP compounds to produce PNP or 3-methyl-*p*-nitrophenol, which could be further

Table 1
Amperometric microbial biosensors.

Target	Microorganism	Working electrode	Detection mode	Limit of detection	Range of detection	Reference
Paraoxon	*P. putida* JS444	Clark oxygen electrode	Batch	55 ppb	0.2–50 μM	Lei et al. (2005a)
Paraoxon	*P. putida JS444*	Carbon paste electrode	Batch	1 nM	Up to 2 μM	Lei et al. (2005b)
Paraoxon	*M.* sp.	Dissolved oxygen electrode	Batch	0.1 μM	Up to 0.05 mM	Mulchandani et al. (2006)
Parathion	*P. putida JS444*	Clark oxygen electrode	Batch	58 ppb	0.2–50 μM	Lei et al. (2005a)
Parathion	*P. putida JS444*	Carbon paste electrode	Batch	1 nM	Up to 2 μM	Lei et al. (2005b)
Methyl parathion	*P. putida JS444*	Clark oxygen electrode	Batch	53 ppb	0.2–50 μM	Lei et al. (2005a)
Methyl parathion	*P. putida JS444*	Carbon paste electrode	Batch	1 nM	Up to 2 μM	Lei et al. (2005b)
Fenitrothion	*P. putida JS444*	Clark oxygen electrode	Batch	277 ppb	Up to 50 μM	Lei et al. (2006b)
Fenitrothion	*P. putida JS444*	Carbon paste electrode	Batch	1.4 ppb	Up to 5 μM	Lei et al. (2007)
EPN	*P. putida JS444*	Clark oxygen electrode	Batch	1.6 ppm	Up to 50 μM	Lei et al. (2006b)
EPN	*P. putida JS444*	Carbon paste electrode	Batch	1.6 ppb	Up to 5 μM	Lei et al. (2007)
Phenol	*P. putida*	Graphite-epoxy composite electrode	Batch	7 μM	8–40 μM	Kirgoz et al. (2006)
Phenol	*E. coli*	Carbon ink electrode	Batch	–	1.6–16 ppm	Neufeld et al. (2006)
Phenol	*P. putida*	CNT modified carbon paste electrode	Batch	–	0.5–4 mM	Timur et al. (2007a)
p-Nitrophenol	*M.* sp.	Carbon paste electrode	Batch	20 nM	Up to 20 μM	Mulchandani et al. (2005)
p-Nitrophenol	*P.* sp.	Oxygen electrode	Batch	–	10–40 μM	Banik et al. (2008)
Catechol	*P. putida*	Cysteamine modified gold electrod	FIA	–	0.025–0.2 mM	Timur et al. (2007b)
Benzene	*P. putida* F1	Dissolved oxygen electrode	FIA	–	0.02–0.14 mM	Rasinger et al. (2005)
Toluene	*P. putida* F1	Dissolved oxygen electrode	FIA	–	0.05–0.2 mM	Rasinger et al. (2005)
Ethylbenzene	*P. putida* F1	Dissolved oxygen electrode	FIA	–	0.1–0.2 mM	Rasinger et al. (2005)
2,4-Dichloro phenoxy acetic acid	*P. putida*	Dissolved oxygen electrode	Batch	–	10–60 μM	Odaci et al. (2009a)
2,4-Dichloro phenoxy acetic acid	*P. putida*	Screen printed graphite electrode	Batch	–	20–80 μM	Odaci et al. (2009a)
Atrazine	*C. vulgaris*	Screen printed carbon electrode	FIA	1 μM	–	Shitanda et al. (2009)
Cu^{2+}	*S. cerevisiae (I)*	Oxygen electrode	FIA	2.1 mg/L	1.6–6.35 mg/L	Tag et al. (2007)
Cu^{2+}	*S. cerevisiae (II)*	Oxygen electrode	FIA	0.0067 mg/L	0.05–0.35 mg/L	Tag et al. (2007)
Fe^{2+}	*A. ferrooxidans*	Oxygen electrode	Batch	0.9 μM	Up to 2.5 mM	Zlatev et al. (2006b)
$S_2O_3^{2-}$	*A. ferrooxidans*	Oxygen electrode	Batch	–	Up to 0.6 mM	Zlatev et al. (2006b)
$Cr_2O_7^{2-}$	*A. ferrooxidans*	Oxygen electrode	Batch	0.4 μM	Up to 0.4 mM	Zlatev et al. (2006c)
BOD	*S. cerevisiae*	Screen printed carbon electrode	Batch	–	6.6–220 mg/L	Nakamura et al. (2007)
BOD	Activated sludge	Clark oxygen electrode	FIA	0.2 mg/L	5–25 mg/L	Kumlanghan et al. (2008)
BOD	Microbial consortium	Clark oxygen electrode	Batch	1 mg/L	Up to 45 mg/L	Dhall et al. (2008)
BOD	*P. syringae*	Dissolved oxygen electrode	Batch	–	5–100 mg/L	Kara et al. (2009)
Glucose	*P. fluorescens*	CNT epoxy composite electrode	Batch	–	0.5–4 mM	Kirgoz et al. (2007)
Glucose	*P. putida*	CNT modified carbon paste electrode	Batch	–	0.05–2 mM	Timur et al. (2007a)
Glucose	*P. putida*	Cysteamine modified gold electrod	FIA	–	1–7.5 mM	Timur et al. (2007b)
Glucose	*P. fluorescens*	PEDOT modified graphite electrode	Batch	–	0.25–4 mM	Odaci et al. (2008a)
Glucose	*P. fluorescens*	Carbon paste electrode	Batch	–	60–750 μM	Yeni et al. (2008)
Glucose	*G. oxydans*	Graphite electrode	Batch	–	0.05–1 mM	Odaci et al. (2009b)
Glucose	*G. oxydans*	Graphite electrode	Batch	–	0.1–2.5 mM	Tuncagil et al. (2009b)
Glucose	*P. fluorescens*	Graphite electrode	Batch	–	0.2–1 mM	Tuncagil et al. (2009a)
Glucose	*G. oxydans*	Graphite electrode	Batch	–	0.25–4 mM	Tuncagil et al. (2009a)
Galactose	*P. putida*	CNT modified carbon paste electrode	Batch	–	0.5–6 mM	Timur et al. (2007a)
Galactose	*P. fluorescens*	Graphite electrode	Batch	–	0.2–1 mM	Odaci et al. (2008b)
Mannose	*P. fluorescens*	Graphite electrode	Batch	–	0.2–1 mM	Odaci et al. (2008b)
Xylose	*P. fluorescens*	Graphite electrode	Batch	–	0.4–1 mM	Odaci et al. (2008b)
Ethanol	*G. oxydans*	Graphite electrode	Batch	–	0.1–5 mM	Tuncagil et al. (2009b)
Ethanol	*C. tropicalis*	Dissolved oxygen electrode	Batch	–	0.5–7.5 mM	Akyilmaz and Dinckaya (2005)
Ethanol	*P. angusta*	Clark oxygen electrode	Batch	0.012 mM	–	Voronova et al. (2008)
Ethanol	*G. oxydans*	Glassy carbon electrode	FIA	3.3 μM	10 μM to 1.5 mM	Valach et al. (2009)
Ethanol	*G. oxydans*	Graphite electrode	Batch	–	0.1–5 mM	Tuncagil et al. (2009a)
1,3-Propanediol	*G. oxydans*	Glassy carbon electrode	FIA	0.15 mg/L	–	Katrlik et al. (2007)
β-D-Glucuronidase	*M.* sp	Glassy carbon electrode	Batch	–	0.2 ng/L to 2 μg/L	Togo et al. (2007)
Vitamin B1	*S. cerevisiae*	Dissolved oxygen electrode	Batch	–	5–100 nM	Akyilmaz et al. (2006)
Choline	*A. globiformis*	Clark oxygen electrode	Batch	80 nM	Up to 0.2 mM	Stoytcheva et al. (2006)
Caffeine	*P. alcaligenes*	Clark oxygen electrode	Batch	–	0.1–1 mg/mL	Babu et al. (2007)
L-lactate	*H. polymorpha*	Graphite electrode	Batch	6 μM	Up to 1.6 mM	Smutok et al. (2007)
L-lysine	*S. cerevisiae*	Dissolved oxygen electrode	Batch	–	1–10 μM	Akyilmaz et al. (2007)
Nalidixic acid	*E. coli*	Micro-chip	Batch	10 μg/mL	–	Ben-Yoav et al. (2009a)
2-Amino-3-methylimidazo[4,5-f]quinoline	*S. typhimurium*	Micro-chip	Batch	0.31 μM	–	Ben-Yoav et al. (2009a)
Methane	*P. aeruginosa* + *K.* sp.	Platinum electrode	Batch	0.3% (v/v)	1–5% (v/v)	Wen et al. (2008)

degraded by the bacteria through the formation of highly electroactive compounds. These highly electroactive compounds could be further reduced or oxidized on a carbon paste electrode (CPE) at an appropriate applied potential to give the current response (Lei et al., 2005b, 2007). On the other hand, the assimilation of PNP by the microbes consumes oxygen. The oxygen concentration change was measured using an amperometric oxygen electrode and then correlated with the concentration of OP compounds (Lei et al., 2005a, 2006b; Mulchandani et al., 2006).

Microbial biosensors have been widely applied in the detection of Biochemical Oxygen Demand (BOD). Traditional measurement of biodegradable organic pollution in wastewater is the off-line 5-day Biochemical Oxygen Demand test (BOD_5) (Bourgeois et al., 2001; Eaton and Franson, 2005). However, this method is time-consuming and not suitable for on-line monitoring (Kara et al., 2009). Microbial biosensors become a promising alternative to rapidly evaluate BOD in the water sample. A BOD microbial biosensor based on highly porous micro-cellular polymer entrapped *Pseudomonas syringae* with an oxygen electrode was constructed and showed a typical response time of 3–5 min (Kara et al., 2009). In order to facilitate the electron transport through the cell and amplify the response, a novel BOD sensor was designed with the eukaryote *Saccharomyces cerevisiae* coupled with two mediators, ferricyanide and menodione (Nakamura et al., 2007). A single strain can only metabolize a narrow substrate spectrum. In order to obviate this limitation, synergetic microbial consortium were applied as the sensing element in the biosensor which demonstrated an attractive detection limit as low as 1 mg/L (Dhall et al., 2008). Flow injection analysis (FIA) enables microbial biosensors to carry out continuous monitoring and on-line detection. A microbial BOD sensor equipped with an oxygen electrode and activated sludge was developed and successfully applied to the real-time monitoring of anaerobic reactors (Kumlanghan et al., 2008). In addition, Velling et al. compared different calibration methods, namely static and dynamic interpretation of the current response, employed in microbial biosensors for BOD detection (Velling and Tenno, 2009). The results revealed that the dynamic approach offered a broader detection area and a quicker response; however, it was more vulnerable to the instability of the system than the static method.

Due to their high toxicity to the environment, phenol and substituted phenolic compounds are categorized as U.S. Environmental Protection Agency (EPA) priority chemicals (Lei et al., 2006a). Therefore, there is an urgent need for innovative analytical tools/devices to facilitate the detection of these compounds in case of an accidental release. Based on the respiratory activity of adapted cells, *P. putida* immobilized graphite-epoxy composite electrode or CPE with carbon nanotubes (CNTs) was applied to phenol detection (Kirgoz et al., 2006; Timur et al., 2007a). In another study, a recombinant *Escherichia coli* strain extremely sensitive to membrane-damaging chemicals was constructed and could detect as low as 1.6 ppm phenol within 20 min (Neufeld et al., 2006). Particularly, a plasmid carrying *fabR* gene which codes for the repressor of the promoter gene was transformed along with the functional gene. This provided a novel strategy to reduce the background noise resulting from the incomplete shutoff of the promoter. On the basis of electron transfer, PNP-degrader, *Moraxella* sp. modified CPE was employed in the fabrication of PNP microbial biosensor (Mulchandani et al., 2005). Based on the respiration of cells, *Pseudomonas* sp. entrapped polycarbonate membrane modified oxygen electrode was also applied to detect PNP which serves as the sole carbon and nitrogen source for the bacteria (Banik et al., 2008). In addition, *Moraxella* and *P. putida* modified glassy carbon electrodes (GCE) were utilized in the detection of *E. coli* in water based on the highly sensitive amperometric detection of PNP, a metabolic product of *E. coli*, with the addition of *p*-nitrophenyl-β-D-glucuronide (Togo et al., 2007).

Heavy metal is another kind of important target analyte in the environment. A whole-cell amperometric sensing system for Cu^{2+} using FIA was developed based on the recombinant *S. cerevisiae* and an oxygen electrode (Tag et al., 2007). *CUPI* gene, serving as the promoter, was fused with the reporter *lacZ* gene. Cu^{2+} switched on the expression of *CUP1*/*lacZ* in tandem. The product of *lacZ* gene (β-galactosidase), which was in proportion to the concentration of Cu^{2+}, could catalyze the oxidation of glucose with the consumption of oxygen. *Acidithiobacillus ferrooxidans*, which is able to directly oxidize Fe^{2+} and $S_2O_3^{2-}$ during its aerobic metabolism, was immobilized on an oxygen electrode to construct an amperometric microbial biosensor for the determination of Fe^{2+} and $S_2O_3^{2-}$ (Zlatev et al., 2006b). Furthermore, on the basis of fundamental redox reaction of $Cr_2O_7^{2-}$ with Fe^{2+}, this biosensor could also be employed to indirectly detect $Cr_2O_7^{2-}$ as low as 0.4 μM by amperometric titration (Zlatev et al., 2006c).

Based on the respiratory activity of the bacteria, target analyte-adapted *P. putida* has been used as the sensing element in the detection of other pollutants, such as BTE (benzene, toluene, ethylbenzene) (Rasinger et al., 2005) and 2,4-dichloro phenoxy acetic acid (Odaci et al., 2009a). The decrease of oxygen consumption rate due to the inhibition of bacterial respiration can also be utilized to detect highly toxic chemicals. According to this mechanism, photosynthetic microalgae *Chlorella vulgaris* modified screen-printed carbon electrode was reported for the continuous evaluation of the toxicity of atrazine and DCMU (Shitanda et al., 2009). In addition, an amperometric biosensor for the quantitative determination of methane has been developed using immobilized mixed culture of *Pseudomonas aeruginosa* and *Klebsiella* sp. along with a dissolved oxygen electrode (Wen et al., 2008). This biosensing system exhibited good reproducibility and high sensitivity with a detection limit of 0.3% (v/v) to methane within 100 s.

Amperometric microbial biosensors also provide rapid and sensitive tools in health and fermentation applications. The detection of glucose, which is of great interest in the diagnosis of diabetes and quality control of fermentation and food, accounts for about 85% of the entire biosensor market (Wang, 2008). Several microbial biosensors for glucose detection have been fabricated based on the oxygen consumption of the respiratory activity in the microbes. The microbial strains serving as the sensing element include *Pseudomonas fluorescens* (Kirgoz et al., 2007; Odaci et al., 2008a, 2008b; Tuncagil et al., 2009a; Yeni et al., 2008) and *Gluconobacter oxydans* (Odaci et al., 2009b; Tuncagil et al., 2009a, 2009b). All the transducers applied here were modified by either CNTs (Kirgoz et al., 2007; Odaci et al., 2008b, 2009b) or CPs (Odaci et al., 2008a; Tuncagil et al., 2009a; Tuncagil et al., 2009b) to enhance the amperometric response of the devices. The working potential for this type of microbial biosensor is around −0.7 V (vs. Ag/AgCl, 3 M KCl). However, many substances are able to be reduced at this potential, resulting in relatively high interference. In order to decrease the absolute value of the working potential, a variety of redox-active molecules (mediator), such as Osmium redox polymers (Timur et al., 2007a; Timur et al., 2007b), ferrocene (Yeni et al., 2008), and 2,5-dibromo-1,4-benzoquinone (Babkina et al., 2006) were applied in the fabrication of glucose microbial biosensors to effectively shuttle the electrons generated in a certain metabolic pathway between the cell wall and the surface of electrode.

Generally, the bacteria, which can uptake glucose, also possess the enzyme activity to metabolize other carbohydrates, such as galactose (Odaci et al., 2008b; Timur et al., 2007a), catechol (Timur et al., 2007b), mannose, and xylose (Odaci et al., 2008b). Selective biosensors can still be developed for different sugars as long as the bacteria are adapted to the specific analyte in advance through the selective cultivation. The qualitative and quantitative detection of alcohols with high sensitivity, selectivity, and accuracy is required in many fields (Azevedo et al., 2005). Several microor-

Table 2
Conductimetric, potentiometric, voltammetric and MFC-based microbial biosensors.

Target	Microorganism	Working electrode	Transducer type	Limit of detection	Range of detection	Reference
Cd^{2+} and Zn^{2+}	*C. vulgaris*	Pt IDE	Conductometric	10 ppb	–	Chouteau et al. (2005)
Cd^{2+}	*C. vulgaris*	Pt IDE	Conductometric	1 ppb	–	Guedri and Durrieu (2008)
Paraoxon-methyl	*C. vulgaris*	Pt IDE	Conductometric	100 ppb	–	Chouteau et al. (2005)
Urea	*B. ammoniagenes*	Pt-twin wire electrode	Conductometric	–	Up to 75 mM	Jha et al. (2009)
Cephalosporins	*P. aeruginosa*	pH electrode	Potentiometric	–	0.1–11 mM	Kumar et al. (2008)
Cephalosporins	*B. stearothermophilus var. calidolactis*	CO_2 electrode	Potentiometric	MRL	–	Ferrini et al. (2008)
Penicillins	*B. stearothermophilus var. calidolactis*	CO_2 electrode	Potentiometric	MRL	–	Ferrini et al. (2008)
Cu^{2+}	*C. sp.*	CPE	Voltammetric	54 nM	0.5–10 μM	Alpat et al. (2008)
Glucose	*bacteria consortium*	Graphite rod (anode)	MFC	25 mg/L	25 g/L	Kumlanghan et al. (2007)
Acetate	*G. sulfurreducens*	Graphite cloth (anode)	MFC	–	Up to 2.3 mM	Tront et al. (2008)
BOD	*anaerobic sludge*	Carbon cloth (anode)	MFC	–	Up to 350 g BOD/L	Di Lorenzo et al. (2009)

ganisms which can metabolize ethanol with the consumption of oxygen, such as *G. oxydans* (Tuncagil et al., 2009a; Tuncagil et al., 2009b), *Pichia angusta* (Voronova et al., 2008), and *Candida tropicalis* (Akyilmaz and Dinckaya, 2005) have been applied to the construction of ethanol whole-cell biosensors. Moreover, a *G. oxydans*-based amperometric biosensor with ferricyanide serving as the mediator for the measurement of ethanol in FIA system was constructed (Valach et al., 2009). The experimental results showed that the response was very stable during 72 h of continuous monitoring and the detection limit was as low as 3.3 μM for ethanol. Another microbial biosensor based on *G. oxydans* for the selective determination of 1,3-propanediol (1,3-D) in the presence of glycerol (Gly) in the flow system was also reported (Katrlik et al., 2007). With the help of either ferricyanide or Osmium redox polymers, a good operational stability was demonstrated by 140 h of continuous operation with a selectivity ratio (1,3-D/Gly) of 118 or 145, respectively.

Microbial biosensors are prospective in the determination of some compounds, which is costly and time-consuming by using traditionally analytical method. *S. cerevisiae* coupled with an amperometric oxygen electrode was shown to be sensitive in the detection of thiamine (Akyilmaz et al., 2006) and L-lysine (Akyilmaz et al., 2007). *Arthrobacter globiformis* (Stoytcheva et al., 2006) and *Pseudomonas alcaligenes* (Babu et al., 2007) modified oxygen electrodes were reported for the determination of choline and caffeine, respectively. A L-lactate selective biosensor was developed using permeabilized, genetically engineered *Hansenula polymorpha* immobilized on a graphite electrode with phenazine methosulphate as the free-diffusing redox mediator (Smutok et al., 2007). Recently, a whole-cell biosensor on the basis of recombinant *Salmonella typhimurium* TA1535 pSK1002 coupled with a screen-printed gold electrode was designed for the amperometric analysis of genotoxicity, and the result obtained therein was consistent with that obtained by standard SOS-umu-test (Buchinger et al., 2010). In another recent study, *E. coli* RFM443/pBR2TTS and *S. typhimurium* TA1535 pSK1002 were integrated onto a microchip to fabricate novel microfluidic whole-cell biosensors for the detection of two genotoxic compounds, nalidixic acid (NA) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), respectively (Ben-Yoav et al., 2009a). The detection limits of 10 μg/mL for NA and 0.31 μM for IQ were achieved.

3.1.2. Other electrochemical microbial biosensors

The other recently published electrochemical microbial biosensors based on conductometric, potentiometric, voltammetric transducers as well as MFC-based biosensors are summarized in Table 2.

The conductometric microbial biosensor is attractive and appealing owing to its fast and sensitive response to the analytes. In this regard, a conductometric biosensor using *C. vulgaris* microalgae as the bioreceptor was constructed to detect heavy metal ions and pesticides in water sample (Chouteau et al., 2005). *C. vulgaris* was immobilized on a bovine serum albumin membrane deposited on a platinum interdigitated electrode (IDE). The developed biosensors were sensitive to Cd^{2+} and Zn^{2+} with a limit of detection of 10 ppb for both ions after a 30 min exposure, while Pb^{2+} did not introduce any significant interference since it would be preferably adsorbed on albumin. The application of the same microbial biosensor in the determination of OP pesticides was also demonstrated as OP compounds can inhibit the activity of acetylcholinesterase. A similar conductometric biosensor using *C. vulgaris* as the sensing element was fabricated to detect Cd^{2+} and the detection limit was as low as 1 ppb Cd^{2+} (Guedri and Durrieu, 2008). Another conductometric biosensor was constructed by entrapping lyophilized *Brevibacterium ammoniagenes* in the pH sensitive polyaniline on a Pt twin wire electrode to detect urea (Jha et al., 2009). The catabolic activity of bacteria produced ammonia from urea, thereby causing an increase of the local pH. The variation pH resulted in the change the resistivity change of the CP, which was detected by the working electrode.

Potentiometric microbial biosensors detect the amount of analytes by measuring the potential difference between the working electrode and the reference electrode separated by a selective membrane. Recently, a potentiometric biosensor based on the pH electrode modified by permeabilized *P. aeruginosa* was developed for selective and rapid detection of cephalosporin group of antibiotics (Kumar et al., 2008). The hydrolysis of cephalosporin, due to the enzyme activity of the microbial layer, was accompanied by the production of protons near the pH electrode. The response came from the change of electric potential difference between the working electrode and the reference electrode. Another potentiometric biosensor for the identification of β-lactam residues in milk was also reported (Ferrini et al., 2008). This analytical device consisted of *Bacillus stearothermophilus* var. *calidolactis* and a carbon dioxide (CO_2) electrode, which measured the content of CO_2 released by the microorganisms. The presence of β-lactams inhibited the microbial growth and, consequently, the CO_2 production rate. In addition, a potentiometric whole-cell biosensor based on ion-sensitive field effect transistor (ISFET) pH electrode has been successfully applied to the detection of *E. coli* K12 bacterial activity using a three-dimensional agarose as the immobilization matrix (Bettaieb et al., 2007).

Voltammetry such as cyclic voltammetry (CV) is another commonly used technique in electrochemical analysis. Recently, a voltammetric microbial biosensor for the determination of Cu^{2+} was reported (Alpat et al., 2008). The biosensor was based on *Circinella* sp. modified carbon paste electrode. Cu^{2+} was preconcentrated on the electrode and measured by CV, which was then interpreted as peak current to determine the concentration of the

target analyte. The detection limit of this biosensor could be as low as 54 nM Cu^{2+}. Furthermore, a modified stripping voltammetry was proposed by taking advantage of both bacterial adsorption on a mercury surface and metal fixation capacity on *A. ferrooxidans* (Zlatev et al., 2006a). The heights of resultant peaks for the detection of different metals were increased 17.6% for copper, 48.4% for lead, and 132% for cadmium compared to those obtained with unmodified mercury electrode.

With its current progress, MFC can also be applied as a biosensor for *in situ* analysis and monitoring target chemicals (Du et al., 2007; Li et al., 2010). A single-chamber, proton exchange membrane (PEM) based MFC was constructed where a replaceable anaerobic consortium was used for continuously analyzing glucose in the anode electrolyte (Kumlanghan et al., 2007). The anaerobes oxidized glucose and transferred electrons to the external circuit. Therefore, the maximum closed circuit potential (equivalent to the maximum current at a constant circuit load) could be correlated with the concentration of glucose in the sample solution. Recently, a MFC biosensor system for the detection of acetate was explored (Tront et al., 2008). *Geobacter sulfurreducens* was used as an external electron acceptor and the system was operated with influent solution containing acetate at various concentrations and monitored for the change of generated current. MFC-based biosensor has also been applied to BOD detection. A MFC biosensor for BOD was constructed and optimized with anaerobic sludge and carbon cloth as the anode (Di Lorenzo et al., 2009). Artificial wastewater was fed as the fuel and the current output had a linear relationship with the BOD concentration up to 350 mg BOD/mL with high reproducibility and operational stability.

3.2. Optical microbial biosensors

Some recently reported optical microbial biosensors are summarized in Table 3.

3.2.1. Fluorescent microbial biosensors

Based on the detection mode, fluorescent microbial biosensors can be divided into two categories: *in vivo* and *in vitro*. *In vivo* fluorescent microbial biosensor makes use of genetically engineered microorganisms with transcriptional fusion between an inducible promoter and a reporter gene encoding fluorescent protein. Green fluorescent protein (GFP), encoded by *gfp* gene, is among the most popular tools due to its attractive stability and sensitivity, and the fluorescence emitted by GFP can be conveniently detected by modern optical equipments with little or no damage to the host system (Pickup et al., 2005). As a rule of thumb, the promoter gene is induced quantitatively by the target analyte, thus the concentration of which can be correlated with the fluorescent intensity of GFP expressed by *gfp* reporter. On the basis of this mechanism, *P. putida* whole-cell biosensor was engineered to determine the bioavailable naphthalene (Kohlmeier et al., 2008). The result obtained by the biosensor has shown great consistency with those from Tenax extraction and chemical analysis but the speed was much faster. Fiorentino et al. developed a microbial biosensor using genetically engineered *E. coli* for measuring aromatic aldehydes in aqueous system (Fiorentino et al., 2009). GFP was expressed under the control of an alcohol dehydrogenase-induced promoter. This biosensor showed a linear response in the millimolar range and was able to differentiate between various aromatic aldehydes. Furthermore, a novel method based on the formation of spores has been used to preserve, store, and transport whole-cell biosensing system that would allow for broad on-site applications (Date et al., 2007). This *Bacillus megaterium* sensing system for the detection of zinc could express enhanced green fluorescent protein (eGFP) under the transcriptional control of the promoter and *smt* operon, with a detection range of 10^{-6} to 10^{-4} M analyte. A bioengineered strain of *Caulobacter crescentus* would become fluorescently green in the presence of toxic levels of uranium (Hillson et al., 2007). In this system, the promoter *urcA* was strongly up-regulated upon the exposure to uranium and the expressed GFPuv could be detected *in situ* with a hand-held UV lamp when the concentration of uranyl cation was higher than 0.5 μM. Additionally, Hever et al. constructed a plasmid that allowed for the quantification of two types of deleterious environment effect: *grpE* promoter fused with the gene coding for red fluorescent protein (RFP) in response to ethanol, and recA promoter with *gfp* gene coding for green fluorescent protein to detect nalidixic acid (Hever and Belkin, 2006). Another type of fluorescent biosensor was based on the construction of *E. coli* auxotroph strain and monitoring the GFP signal as an indicator of growth rate. A lysine auxotroph strain *E. coli* has been used to determine the total and bioavailable lysine contents in different feed ingredients and complete feed (Chalova et al., 2007, 2008). These results were reliable and accurate because they were in good agreement with those obtained from high performance liquid chromatography (HPLC), chick bioassay, or other traditional methods. Similarly, a genetically engineered mevalonate auxotroph *E. coli* was constructed to observe the mevalonate concentration in the growth medium (Pfleger et al., 2007). Flow cytometry is able to rapidly distinguish bacteria that elicit fluorescence from complex mixtures of particles and quantify the level of fluorescence in each individual bacterium. Based on this technique, Bahl et al. improved a tetracycline *E. coli* biosensor by inserting the *tet*(M) resistance gene into *E. coli*, and demonstrated an extended detection range of 5 ng/mL to 16 μg/mL (Bahl et al., 2005). This group also selected an indigenous soil bacterium, belonging to the family of *Enterobacteriaceae*, and engineered it with acylated homoserine lactones (AHLs)-regulated *luxI* promoter and *gfp* reporter gene (Burmolle et al., 2005). This whole-cell biosensor determined that AHL compounds were produced during the degradation of litter in soil. Additionally, flow cytometry and extended pre-incubation were combined to improve SOS-GFP assay for the detection of alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) in solution and of genotoxins mitomycin C in soil (Norman et al., 2006). Besides aforementioned fluorescent microbial biosensors, Martineau et al. reported a miniature flow-through optical cell-based disposable fluorescent microbial biosensor for the detection of genotoxin mitomycin C (Martineau et al., 2009). As reporter proteins, GFP and RFP were fused with the *rec* promoter and the corresponding plasmid was transformed into *E. coli*. The exposure of the genetically engineered *E. coli* to the DNA-damaging agent mitomycin C activated the *rec* promoter, which could be quantified by the production of fluorescent reporters. The limit of detection for mitomycin C was estimated to be as low as 0.25 nM for *recA::mCherry* and 2.0 nM for *recA::gfpmut3.1*, respectively. By combining GFP and RFP reporter proteins into one cascade, the same group constructed a two-stage amplifying promoter cascade (*recA::gfpmut3.1-LacI-tac::mCherry*) which offered an enhanced sensitivity and a unique optical density-free assessment mode (Martineau et al., 2010). In this dual reporter cascade, DNA-damage activated the *rec*A promoter, thus GFP and *LacI* protein were up-regulated. Furthermore, *LacI* represses the *tac* promoter, resulting in the down-regulation of RFP. Compared to their previously reported single reporter system, the dual reporter construct had an improved limit of detection of 0.1 nM mitomycin C.

Some *in vitro* fluorescent whole-cell biosensors have been designed and successfully applied to monitoring environmental pollutants. A fluorescent BOD sensor by immobilizing mixed culture of microorganisms and a dissolved oxygen optical fiber in ormosil–PVA matrix was constructed and exhibited improved performance compared to the traditional BOD_5 method (Lin et al., 2006). The fluorescent intensity of the oxygen-sensing film changed with the consumption of oxygen by the immobilized bacteria with the addition of water sample. The as-prepared biosensors could

Table 3
Optical (fluorescent, bioluminescent, and colorimetric) microbial biosensors.

Target	Microorganism	Promoter::reporter	Transducer type	Limit of detection	Reference
Lysine	*E. coli* ΔlysA mini-Tn5-gfpmut3	–	Fluorescent	–	Chalova et al. (2007)
Lysine	*E. coli ΔlysA mini-Tn5-Km-gfpmut3*	–	Fluorescent	3 μg/ml	Chalova et al. (2008)
Tetracycline	*E. coli MC4100/pTGM*	*Ptet(M)::tet(M) Ptet(A)::gfp*	Fluorescent	5 ng/mL to 16 μg/mL	Bahl et al. (2005)
Naphthalene	*P. putida pPG7gfp*	–	Fluorescent	–	Kohlmeier et al. (2008)
Toluene	*P. putida mt-2, P. putida F1, P. mendocina KR1, and B. cepacia G4*	*resazurin respiration*	Fluorescent	0.2 mM	Tizzard et al. (2006)
Toluene (bioavailable BTEX)	*E. coli* DH5α pTOLGFP	*Pu::gfp*	Fluorescent	25 μM	Li et al. (2008)
Aromatic aldehydes	*E. coli BL21DE3(RIL)*	*Sso2536adh::egfp*	Fluorescent	–	Fiorentino et al. (2009)
Nalidixic acid and ethanol	*E. coli NHEX-R*	*recA′::gfpuv grpE′::DsRed*	Fluorescent	–	Hever and Belkin (2006)
Durion (herbicide)	*C. vulgaris*	–	Fluorescent	1 μg/L for	Nguyen-Ngoc and Tran-Minh (2007)
Uranium	*C. crescentus NJH371*	*urcA::gfpuv*	Fluorescent	0.5 μM	Hillson et al. (2007)
Mevalonate	*E. coli DP5*	*lac::gfp*	Fluorescent	–	Pfleger et al. (2007)
BOD	*B. licheniformis, D. maris and M. marinus from sea water*	*oxygen optical fiber*	Fluorescent	0.2 mg/L	Lin et al. (2006)
Zinc	*B. megaterium pSD202*	*smt::egfp*	Fluorescent	1 μM	Date et al. (2007)
N-Methyl-N′-nitro-N-nitrosoguanidine (MNNG)	*E. coli MG1655*	*pANO1::cda′*	Fluorescent	5 nM	Norman et al. (2006)
Acylated homoserine lactones	*Enterobacteriaceae MB101/pJBA132*	*luxI::gfp(ASV)*	Fluorescent	–	Burmolle et al. (2005)
Mitomycin C	*E. coli MG1655*	*pANO1::cda′*	Fluorescent	2.5 ng/g soil	Norman et al. (2006)
Mitomycin C	*E. coli MG 1655 Δ(argF-lac)U169*	*recA::mCherry*	Fluorescent	0.25 nM	Martineau et al. (2009)
Mitomycin C	*E. coli MG 1655 Δ(argF-lac)U169*	*recA::gfpmut3.1*	Fluorescent	2.0 nM	Martineau et al. (2009)
Mitomycin C	*E. coli MG 1655 Δ(argF-lac)U169*	*recA::gfpmut3.1-LacI-tac::mCherry*	Fluorescent	0.1 nM	Martineau et al. (2010)
Toxicity of Heavy metals	*A.* sp. *DF4/PUTK2*	*lux*	Luminescent	–	Abd-El-Haleem et al. (2006)
Genotoxicity of herbal tinctures	*E. coli DPD2794*	*recA::lux*	Luminescent	–	Watt et al. (2007)
General oxidative stress of herbal tinctures	*E. coli DE135*	*uspA::lux*	Luminescent	–	Watt et al. (2007)
Methyl viologen	*E. coli PGRFM*	*pgi::lux*	Luminescent	0.6–19.3 ppm (nutrient-limited); 4.9–625 ppm (nutrient-rich)	Niazi et al. (2008)
Toxicity of individual BTEX	*P. putida F1 Tn5*	–	Luminescent	–	Dawson et al. (2008)
Bioavailable BTEX	*P. putida TVA8*	*tod::lux*	Luminescent	–	Dawson et al. (2008)
Toluene (bioavailable BTEX)	*E. coli DH5α pTOLLUX*	*Pu::lux*	Luminescent	7.5 μM	Li et al. (2008)
Tetracycline	*E. coli K12 pTetLux1*	*tetA::lux*	Luminescent	25 ng/g tissue	Virolainen et al. (2008)
Disinfection efficacy of chlorine dioxide	*P. fluorescens 5RL*	*sal::lux*	Luminescent	–	del Busto-Ramos et al. (2008)
Doxycycline (tetracyclne residue)	*E. coli K12 pTetLux1*	*tetA::lux*	Luminescent	5 ng/g tissue	Virolainen et al. (2008)
Chlortetracycline (tetracyclne residue)	*E. coli K12 pTetLux1*	*tetA::lux*	Luminescent	7.5 ng/g tissue	Virolainen et al. (2008)
Oxytetracycline (tetracyclne residue)	*E. coli K12 pTetLux1*	*tetA::lux*	Luminescent	25 ng/g tissue	Virolainen et al. (2008)
Environmental toxicity	*E. coli DPD2794*	*lac::lux*	Luminescent	–	Elman et al. (2008)
2,4-Dichlorophenol	*V. fischeri*	*lux*	Luminescent	40–200 mg/L	Stolper et al. (2008)
3,4-Dichlorophenol	*V. fischeri*	*lux*	Luminescent	7–90 mg/L	Stolper et al. (2008)
3,5-Dichlorophenol	*V. fischeri*	*lux*	Luminescent	30–100 mg/L	Stolper et al. (2008)
2,3,5,6-Tetrachlorophenol	*V. fischeri*	*lux*	Luminescent	4–30 mg/L	Stolper et al. (2008)
2,3,4,6-Tetrachlorophenol	*V. fischeri*	*lux*	Luminescent	5–40 mg/L	Stolper et al. (2008)
Biodegradation of naphthalene in soil	*P. fluorescensd HK44 pUTK21*	*lux*	Luminescent	0–15 mg/L	Paton et al. (2009)
Chloramphenicol	*E. coli EMS310*	*cspA::luc*	Luminescent	1 μg/mL	Shapiro and Baneyx (2007)
Ofloxacin	*E. coli EMS500*	*sulA::lucR1*	Luminescent	0.05 μg/mL	Shapiro and Baneyx (2007)
Simultaneous detection of chloramphenicol and ofloxacin	*E. coli MULTILacZ*	*sulA::lucR1 cspA::luc*	Colorimetric + luminescent	–	Shapiro and Baneyx (2007)
Arsenite	*R. sulfidophilum CDM 2*	*O/Pars arsR::crtA*	Colorimetric	3 μg/L	Fujimoto et al. (2006)
Delor 103 (polychlorinated biphenyls)	*P.* sp. *P2*	–	Colorimetric	0.5 mg/L	Gavlasova et al. (2008)
2,4,4′-Trichlorobiphenyl (polychlorinated biphenyls)	*P.* sp. *P2*	–	Colorimetric	0.2 mg/L	Gavlasova et al. (2008)
Methyl parathion	*F.* sp.	–	Colorimetric	0.3 μM	Kumar et al. (2006)

detect BOD in the range of 0.2–40 mg/L and had a shorter response time and better storage stability. Chlorophyll has been employed as a fluorescent compound for anti-PSII herbicide detection (Nguyen-Ngoc and Tran-Minh, 2007). Vegetal whole cells *C. vulgaris* were immobilized in an inorganic translucent sol-gel matrix. Chlorophyll could be metabolized by photosystem II (PSII) activity in the microbes. The presence of anti-PSII herbicide (e.g. diuron) would inhibit the electron transfer in the PSII and result in an increase of chlorophyll fluorescence emission. Another fluorescent biosensor was based on the catabolism of contaminant compounds by specific bacterium and concomitantly reducing the redox indicator (e.g. resazurin) to provide the fluorescent signal in a whole-cell biosensor. The formation of reducing equivalents was enhanced in the assay when a recognized analyte entered the catabolic pathway, resulting in an increased fluorescent intensity (Tizzard et al., 2006). On the basis of this concept, toluene-degrading bacteria along with redox indicator resazurin were employed as the sensing element to detect toluene pollutant and a detection limit of 0.2 mM was achieved.

3.2.2. Bioluminescent microbial biosensors

A bioluminescent microbial biosensor measures the luminescence change emitted by living microorganisms. The luminescence change is, in fact, caused by *lux* gene-coded luciferase responding to the target analyte in a dose-dependent manner. The expression of *lux* gene in microorganisms can be controlled in either a constitutive or inducible manner.

In the constitutive manner, the *lux* gene exists constitutively in the sensing microbe and the bioluminescence will change directly with the addition of chemicals of interest. Based on the fact that the light intensity produced by the bacteria could be reduced in the presence of toxic compounds, a *Vibrio fischeri*-based bioluminescent microbial biosensor was developed for the rapid determination of the toxicity of some common environmental pollutants in a continuous flow system (Stolper et al., 2008). Another bioluminescent microbial biosensor based on *luxCDABE*-marked *Acinetobacter* sp. bacterium was used to assay the toxicity of wastewater contaminated by heavy metals (Abd-El-Haleem et al., 2006). In addition, a bioluminescent biosensor with *P. fluorescens* HK44 pUTK21 recognition element was designed to reflect the available fraction of naphthalene in soil since there was a linear relationship between the luminescence from microbes and the naphthalene concentration (Paton et al., 2009).

In the inducible manner, the reporter *lux* gene is fused with a promoter gene regulated by the existence and the concentration of the target analyte. Compared to the constitutive manner, inducible manner provides a more sophisticated and specific detection of a certain compound. A novel oxidative stress-responsive whole-cell biosensor was constructed using the recombinant *E. coli* PGRFM containing *luxCDABE* reporter fused with *pgi* promoter which could be induced in a dose-dependent manner by methyl viologen (Niazi et al., 2008). A tetracycline (TC) luminescent whole-cell biosensor was developed for the rapid and specific TC residue assay in poultry muscle tissue with membrane permeabilizing agent polymyxin B and sensitizing agent EDTA (Virolainen et al., 2008). *Photorhabdus luminescens* bacterial luciferase operon *luxCDABE* was inserted as an *Eco*RI fragment under the control of TC-inducible *tetA* promoter. The biosensor was shown to be sensitive to TCs at the concentration as low as the EU maximum residue limit. Similarly, a *P. putida*-based biosensor containing *lux* reporter fused with BTEX-specific *tod* promoter was reported to assess the biodegradion of BTEX (benzene, toluene, ethylbenzene and xylene) in soil over time (Dawson et al., 2008). Another bioavailable BETX biosensor using recombinant *E. coli* comprised of *luxCDABE* reporter and *Pu* and compared with the strain using *gfp* reporter gene (Li et al., 2008). The results showed that bioluminescence provided faster and more sensitive detection of toluene and related compounds than fluorescence did. In order to evaluate the antibacterial actions of herbal tinctures, two *E. coli* luminescent biosensors were constructed using *lux* gene fused with two different promoters, *recA* and *uspA*, which were specifically responsive to DNA damage and general oxidative stress, respectively (Watt et al., 2007). Any light emitted on challenging the biosensors with an herbal extract indicated a DNA damage or general toxic event. The inhibition of the target analyte to the bioluminescence can also be applied in this type of biosensor. A prototype luminescent biosensor based on *luxCDABE* reporter regulated by *sal* promoter was designed to estimate the antimicrobial efficacy of chlorine dioxide (ClO_2) (del Busto-Ramos et al., 2008). *P. fluorescens* 5RL, serving as the sensing element, had the ability to produce high levels of luminescence when growing in the presence of salicylate. Exposure of the biosensor to ClO_2 would result in a fast decrease of the luminescence in a dose-dependent manner.

Eukaryotic *Photinus pyralis* luciferase, encoded by *luc* gene, has also been under intensive research and successfully served as a reporter gene in a variety of applications (Greer and Szalay, 2002). Recently, a *cspA::luc*-based whole-cell biosensor was designed to detect chloramphenicol, which is a kind of C-group translational inhibitor, and has shown to be faster and more sensitive than *cspA::lacZ*-based colorimetric biosensor (Shapiro and Baneyx, 2007). Shapiro et al. isolated *P. pyralis* luciferase mutants which could emit light in the red-orange region rather than yellow-green region as wild type does (Shapiro et al., 2005). One *luc* gene of the variants (*lucR1*) was fused with ofloxacin induced *sulA* promoter to detect DNA-damaging agents (Shapiro and Baneyx, 2007). In addition, the *sulA::lucR1* fusion could be combined with a *cspA::lacZ* fusion to yield a dual mode (bioluminescent-colorimetric) biosensor for the simultaneous detection of C-group translational inhibitor and DNA-damaging agents (Shapiro and Baneyx, 2007).

Furthermore, a micro-opto-electro-mechanical-system (MOEMS) modulator and solid-state photo-detectors were implemented in a lab-on-chip whole-cell biosensor on the basis of genetically engineered *E. coli* harboring *luxCDABE* reporter fused with *lac* promoter (Elman et al., 2008). Low intensity optical signals were detected in response to IPTG concentrations of 0.1, 0.05, and 0.02 mM. This preliminary result revealed the feasibility of this technique as an inexpensive approach for rapid environmental toxicity detection. In a simulation study, Ben-Yoav et al. established a 3D optical model of a bioluminescence whole-cell biochip and optimized the optical aspects with respect to bacterial concentration and system geometry, which would help to design a more efficient light collecting biochip (Ben-Yoav et al., 2009b).

3.2.3. Colorimetric microbial biosensors

Colorimetric microbial biosensors involve the generation of colored compound which can be measured and correlated with the concentration of analytes. Recently, a whole-cell colorimetric biosensor was constructed for the detection of arsenite with high sensitivity (Fujimoto et al., 2006). Briefly, photosynthetic bacterium, *Rhodovulum sulfidophilum*, synthesizes carotenoids through the spheroiden (SE) pathway where the yellowish SE is catalyzed by SE monooxygenase (CrtA) to form reddish spheroidenone which is the predominant carotenoid under semi-aerobic conditions. In this biosensor, a genetically engineered photosynthetic bacterium, *crtA*-deleted *R. sulfidophilum* was used as the host strain which accumulated SE and hence displayed yellow color. On the other hand, the arsenite resistance gene cloned from *E. coli* consisted of an operator/promoter region (*O*/*Pars*) and a repressor (ArsR). The presence of arsenite caused the dissociation of ArsR from the operator. Therefore, the biosensor plasmid containing *crtA* promoter fused with *O*/*Pars* and *arsR* operon was transformed into the host strain. In the presence of arsenite, the color change of

the biosensor from yellow to red could be recognized by naked eye and the limit of detection was reported to be 5 μg/L arsenite. Furthermore, several green mutant host strains were developed in such colorimetric biosensor based on the same mechanism since the change in bacterial color from green to red was more distinguishable than that from yellow to red (Yoshida et al., 2007). A more dedicated spectrophotometer is commonly applied in the construction of colorimetric microbial biosensors since some chromophoric compound is not so distinguishable by the naked eye, but its absorbance can be facilely measured by spectrophotometer at certain wavelength (λ). Recently, whole cells of *Flavobacterium* sp. were entrapped in glass fiber filter and applied, along with the optic fiber system, to the detection of methyl parathion pesticide (Kumar et al., 2006). This disposable colorimetric biosensor was based on the OPH activity of *F.* sp., which is able to hydrolyze methyl parathion into PNP. PNP was then quantified by measuring its absorbance at λ = 400 nm. The limit of detection was 0.3 μM methyl parathion, comparable with that of amperometric approach. In addition, the result obtained from the biosensor was in good agreement with that obtained by gas chromatography method. Another whole-cell optical biosensor using the silica immobilized *P.* sp. P2 was reported for the detection of polychlorinated biphenyls with three chlorine substitutions (Gavlasova et al., 2008). The detection principle was based on the measurement of yellowish products with characteristic wavelength at 398 nm. The biosensor also demonstrated good repeatability and storage stability.

4. Conclusions and future trends

Microbial biosensors have been under extensive investigation over decades. Particularly, some electrochemical and optical microbial biosensors developed for environmental applications have been commercialized. For example, commercial on-line BOD microbial biosensors were available from Biosensores SL Moncofar, Spain and Isco GmbH, Gross Umstadt, Germany. The GreenScreen Environmental Monitoring (EM) with a yeast cellular sensing element was designed for the simultaneous detection of genotoxicity and cytotoxicity by Gentronix Ltd., Manchester, UK. In addition, amperometric and bioluminescent whole-cell toxicity biosensors have been developed by Euroclone Ltd., West Yorkshire, UK and Remedios Ltd., Aberdeen, UK (Farre et al., 2005).

Nevertheless, commercial microbial biosensors are just tips of the iceberg compared to the great amount of academic research on them. The intrinsic disadvantages (slow response, low sensitivity, and poor selectivity) using microorganisms as the biosensing element limit the widespread interest of microbial biosensors on the market. Fortunately, with the development of biotechnology, micro-/nano-technology, and novel immobilization strategy in the past years, microbial biosensors are becoming more powerful in the field of analytical chemistry.

Microbial biosensors typically suffer from the poor selectivity because of the non-specific cellular response to substrates. With the development of biotechnology and the availability of genome sequence for more microorganisms, we can genetically engineer microbes with specific metabolic pathways up-regulated or down-regulated, thus providing enhanced selectivity to specific targets. Another way to improve the selectivity of microbial biosensors is to develop microbial sensor arrays. The introduction of analyte to the microbial sensor arrays will generate a finger-printed response pattern. By combining with artificial neural network analysis, the target compound can be identified.

With the development of nanotechnology, nanostructured materials have been applied in biosensing due to their good biocompatibility, enhanced electron transfer property, and high surface area. Co-immobilization of microbes and nanomaterials (e.g. metal oxide nanomaterials) or immobilization of microbes on nanostructured electrodes could potentially improve the sensitivity of microbial biosensors. In addition, microfabrication has been become a routine work in the laboratory. The use of micron-sized electrodes to develop single microbe-based devices would provide the insight of how microbe responds to various targets, which has many potential applications in drug screening, disease diagnosis, and cellular biology. Furthermore, with the development of lab-on-a-chip technique, the integration of a microbe onto a microfluidic chip to develop a biotic-microelectromechanical system would provide a novel research aspect in the field of microbial biosensors because such systems only require minimal amounts of sample and potentially offers promising analytical characteristics such as fast response and good sensitivity in the detection of analytes.

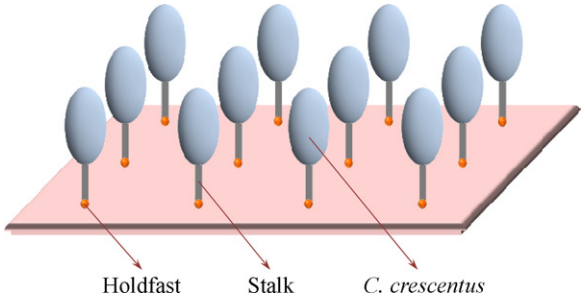

Fig. 1. Schematic of *C. crescentus* monolayer immobilized on the electrode through its very unique adhesive holdfast.

To fabricate a microbial biosensor, microorganisms should be immobilized on transducers or support matrices either by chemical methods (covalent binding and cross-linking) or by physical methods (adsorption and entrapment). However, current immobilization methods have their limitations. Covalent binding and cross-linking greatly affect cell viability as well as cell function. Physical adsorption suffers from poor stability because of the desorption of microbes. A major disadvantage of entrapment immobilization is the additional diffusion resistance caused by the entrapment material, which would result in lower sensitivity. How to simply and successfully immobilize microbial cells without affecting their properties has become a critical step toward the fabrication of a sensitive and cost-effective microbial biosensor. Unlike other microbes, *Caulobacter* species can tightly attach to a surface and form a high density monolayer through its very unique adhesive holdfast positioned at the tip of its stalk. We can take advantage of this feature and develop a simple immobilization method to achieve a bacterial monolayer with very strong adhesion to a surface and without losing any of its biological function (Fig. 1). This will be a breakthrough in the microbial immobilization and will begin a new era for microbial biosensors. Furthermore, the genome sequence of *Caulobacter* is available and its intracellular and surface expressions of protein or enzyme have been realized. Therefore, the functional enzyme or protein can be engineered to *Caulobacter* intracellularly or on its surface, offering an excellent microbial sensing system.

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