Annual Research & Review in Biology
4(17): 2663-2674, 2014

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Whole Cell-based Biosensors for Environmental Heavy Metals Detection

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Review Article

Received 11th February 2014
Accepted 9th April 2014
Published 5th May 2014

ABSTRACT

Biosensors have emerged as new alternatives in environmental toxicity assessment. In the development of biosensors for heavy metals detection in environment, whole cells are highly favored as these cells are able to reflect the real toxicity effects of heavy metals to living organisms. For heavy metals detection, the integration of several types of cells such as bacteria, cyanobacteria, and algae into biosensors development has been widely reported. The usage of other cells such as plant cell, protozoa, and yeast has been reported as well. Although these biosensors are highly sensitive to heavy metals, the detection is still limited to the heavy metals which are bioavailable to the cells. Besides, the response of whole cells to wide range of heavy metals makes them excellent tools for wide spectrum screening but lack of specificity in detection. Whole cells are living entities with complex biochemical processes, which make the optimization of whole cell-based biosensors a tedious process, while maintaining the stability and storability are still challenging tasks. Although naturally occurring cells are highly favored, some reports show that recombinant cells can be a choice with better performance. In this paper, the usage of whole cells in biosensors for heavy metals detection and some of the current issues which are tied to the development of these biosensors are reviewed.

Keywords: Whole cell; biosensor; heavy metals; environment toxicants.

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1. INTRODUCTION

Biosensors are commonly defined as analytical tools with the integration of biological materials such as enzymes, antibodies, organelles, nucleic acids, cells and tissues to electronic devices, intermediated by transducers. However, with a broader view, a biosensor can be any device that can be used to transform certain biological process into signal which later can be read and recorded. Starting with electrode-based biosensor in the early development [1-3], biosensors have broaden the spectrum, from quantitative to qualitative, from simple colour changing strip or single electrode to the usage of the state-of-the-art machines, from single exposure to continuous monitoring tools, and from the biological components extracted from living entities to the synthetic non-living molecules. To extend the practicality of biosensors, the characteristics such as rapid detection, cost effective, high sensitivity, simple operation, and portability have been focused in the development of biosensors, especially for environmental applications [4,5].

For the assessment of environmental toxicants, whole cell biosensors are still in the mainstream of research, as a cell is the simplest entity that can reflect the real physiological effects of the toxicants to the living organism [6,7]. The toxicity effects can then be generalized to bigger and more complex organisms. Besides, cell~~s~~ can be produced or grown easily, thus giving it the financial advantage over other biological components such as enzyme~~s~~ and antibody [8].

To date, different types of whole cells, such as cyanobacteria [9], algae [10], yeast [11], fungi [12], and plant cells [13] have been used in whole cell biosensors. In this review, we would like to focus our discussion on the biosensors which the cells are coupled to electronic devices through transducers [14], while the practicality of the biosensors, such as sensitivity, linear detection range, specificity, lowest limit of detection (LLD), and immobilization are described. Table 1 shows some examples of different types of cells used in biosensors for heavy metals detection in environment.

2. BACTERIAL-BASED BIOSENSORS

Bacteria are highly favored by scientist in the development of environmental biosensors. The microorganisms have high versatility that can strive in various adverse conditions such as extreme temperature, different salinity, pH and even in the environment with the presence of heavy metals. The heavy metal sensitive genes in bacteria makes the microbes excellent candidates for heavy metals detection [17, 28]. A biosensor was constructed by Verma and Singh [18] with *Bacillus sphaericus*, which the enzyme urease synthesized within the cell was used as the reporter for nickel (Ni) detection. The inhibition of the enzyme was used to quantify the concentration of Ni. Oh et al. [29] utilized the sulfur oxidizing ability in sulfur-oxidizing bacteria to detect the presence of chromium (Cr), while the growth and metabolism rate of *Staphylococcus aureus* has been utilized by Sochor et al. [30] in cadmium (Cd) detection.

Table 1. Examples of the whole cell-based biosensors developed for the detection of heavy metals in environment. LLD represents Lowest Limit of Detection in µg/L (unless stated otherwise) and LDR represents Linear Detection Range in µg/L (unless stated otherwise). The sign "-" represents unavailable data

Species	Type of transducer	Heavy metals and media	LLD	LDR	Technique of immobilization	Reference
Bacteria						
Escherichia coli with *golTSB* operon from *Salmonella enterica* and *lacZ* reporter gene	Optical-Colorimetry	Gold (Au) in soil	2	20 - 1000	No immobilization. Cell suspension used	[15]
E. coli with *ars* regulatory element and *Photobacteria luxCDABE* operator-promoter	Optical-Luminescence	Arsenic (As) in water	N/A	0.74 - 60.00	No immobilization. Cell suspension used	[16]
Caulobacter crescentus with GFPuv reporter gene under the control of *Caulobacter urca* gene	Optical- Fluorescence	Uranium (U) in soil and water & food	0.5 µM	-	No immobilization. Sprayed directly onto soil or water surfaces.	[17]
B. sphaericus	Electrochemical-Potentiometry	Ni in water	-	0.002 - 0.040	Physical adsorption onto filter paper.	[18]
Pseudomonas putida with *cadR* promoter fused to *lacIq* and *gfp,* with additional tac promoter and *cadR* transcribed divergently	Optical-Fluorescence	Cd in water	0.01 µM	-	No immobilization. Cell suspension used.	[19]

Table 1 Continued…………

Alcaligenes eutrophus (AE1239) with *Vibrio fischeri luxCDABE* operon under influence of copper induced promoter	Optical-Bioluminescent	Copper (Cu) in water	1 µM	0 - 25 µM	Immobilization in alginate beads	[20]
Cyanobacteria						
Anabaena torulosa	Electrochemical-Amperometry	Cu in water	-	300 - 1000	Entrapment with poly(hydroxyethyl-methacrylate) (pHEMA)	[21]
A. torulosa	Optical- Fluorescence	Cu	1.195	2.5 - 10.0	Entrapment on cellulose membrane	[9]
		Lead (Pb)	0.100	0.5 - 5.0		
		Cd in water	0.027	0.5 - 10.0		
A. torulosa	Optical-Fluorescence	Cu	1.410	2.5 - 10.0	Entrapment on cellulose membrane followed by pHEMA gel entrapment.	[22]
		Pb	0.500	1.0 - 7.5		
		Cd in water	0.250	0.5 - 5.0		
Anabaena flos-aquae	Electrochemical-Amperometry	Cu	-	-	Entrapment with pHEMA	[23]
		Pb in water	-			
Synechocystis sp. PCC 6803 with luciferase (*luxAB*) *reporter* gene and Co and Zn inducible *coaT* promoter or Ni inducibLe *nrsBACD* promoter	Optical-Bioluminescent	Cobalt (Co)	-	0.3 - 6.0 µM	Cell cultures are used and tested directly.	[24]
		Zinc (Zn)		1.0 - 3.0 µM		
		Ni in water		0.2 - 6.0 µM		

Table 1 Continued…………

Algae						
Chlorella vulgaris	Electrochemical-Conductometry	Cd Co Ni Pb Zn in water	1 1 1 1 10	-	Sol-gel silica matrix	[25]
C. vulgaris	Electrochemical-Conductometry	Cd Zn Pb in water	10 10 -	-	Bovine serum albumin reticulated with glutaraldehyde vapours	[26]
C. vulgaris	Electrochemical-Conductometry	Cd in water	1	-	Bovine serum albumin reticulated with glutaraldehyde vapours	[27]
C. vulgaris	Electrochemical-Amperometry	Mercury (Hg) in water	10^{-14} M	10^{-14} M - 10^{-6} M	Algae-bovine serum albumin cross linked with glutaraldehyde	[10]
Others						
Daucus carota	Optical-Spectrometry	Cu Pb Zn in water	10 100 10	10 - 1000 100 - 1000 10 - 1000	No immobilization. Cell suspension used	[13]

The advance in genetic engineering and recombinant DNA technology enable the construct of recombinant bacteria with modified cell metabolic pathway which can produce reliable signals in the presence of targeted analytes [6,8,31]. Ravikumar et al. [32] used recombinant *E. coli* to produce a heavy metal biosensor for Zn and Cu. Ivask et al. [33] reported the usage of 19 recombinant luminescent bacteria biosensor to detect the presence of heavy metals, while Hilson et al. [17] reported the development of U biosensor using *C. crescentus* with the fusion of U sensitive *urcA* gene with fluorescence reporter gene, which allows the quantification of U under UV illumination. In another report, Zammit et al. [15] fused Au sensitive gene *goITSB* which could be found in *Salmonella enterica serovar typhimurium* and *lacZ* reporter gene together and transformed the genes into *E. coli*, thus allowing the quantification of Au through colorimetric test on β-galactosidase. Sharma et al. [16] reported the construction of another *E. coli* biosensor for As detection, which arsenic tolerance genes were chosen as the receptors and fluorescence gene *luxCDABE* was utilized as the reporter.

3. CYANOBACTERIA-BASED BIOSENSORS

Cyanobacteria are found to be blue and green in colour with the size comparable to bacteria. The presence of photosynthetic apparatus similar to higher plants makes these naturally occurring microorganisms suitable to be used in biosensors with the detection parameters focused on photosynthesis related processes and bioenergetics disruption. The amperometric design utilized the change of oxygen level due to the presence of heavy metals, while the optical approach uses the change of fluorescence emission due to the disruption of photosynthesis pathway by heavy metals. Cyanobacteria *A. torulosa* and *A. flos-aquae* had been successfully coupled with optical and amperometric transducers respectively for the detection of Cu, Pb, and Cd [21-23]. *Spirulina subsalsa* was coupled with amperometric transducer for the detection of Cu and Hg [34], while *Nostoc muscorum* and *Synechoccus* PCC 7942 were coupled with optical fluorometric transducer for the detection of Hg and Cd [35].

Apart from photosynthesis related parameters, Awasthi [36] utilized the cyanobacteria cell containing alkaline phosphatase as reporting enzyme in *Anacystis nidulans* for the detection of Ni, Zn, and Cd. The quantification of heavy metals was done through the reporter p-nitrophenol produced by alkaline phosphatase. Another cyanobacteria *Arthrospira platesis* was reported to be able to produce alkaline phosphatase as reporter as well [37]. Similar to bacteria, researchers could produce genetically engineered cyanobacteria for biosensor application. Peca et al. [24] utilized recombinant DNA techniques to fuse heavy metal inducible promoter with luciferase (*luxAB*) reporter gene into *Synechocystis* sp. to detect Co, Zn, and Ni. These heavy metals can be quantified through optical transducer by measuring the change in bioluminescence intensity.

4. ALGAE-BASED BIOSENSORS

Apart from cyanobacteria, algae are highly sensitive to environmental pollutants [38,39]. Microalgae are more common to be used in biosensor applications due to the microscopic size that ease culturing, immobilization and have high reproductive rate.

One of the most common algae species used in biosensors was *C. vulgaris.* It was widely used due to the presence of several enzymes around the extracellular membrane which could act as reporter elements in the presence heavy metals. One of these enzymes is alkaline phosphatase [26]. Durrieu et al. [40] designed a biosensor based on inhibitory action of heavy metals towards alkaline phosphatase on *C. vulgaris*. The quantification was carried out optically by measuring the fluorescence emission from methylumbelliferone (MUF) produced when the added methylumbelliferoyl phosphate (MUP) reacted with the residual of reactive alkaline phosphatase.

Electrohemical transducer has been coupled with alkaline phosphatase as well. Chouteau et al. [26, 27] reported the use of conductometric electrodes to detect the changes in conductivity induced by the catalytic reaction of the enzyme after the exposure to Cd. In addition, conductometric micro transducer was also used by Berezhetskyy et al. [25] to detect Cd, Cu, Ni, Pb, and Zn. The activity of alkaline phosphatase that dephosphorylates p-nitrophenyl phosphate into p- nitrophenol and phosphate ions had been used by Singh and Mittal [10] to design an amperometric biosensor that detects the current produced by the electroactive p-nitophenol, thus allowing detection of heavy metals through its inhibitory action on alkaline phosphatase.

5. OTHER TYPES OF WHOLE CELL-BASED BIOSENSORS

Tetrahymena thermophile- a ciliated protozoan was introduced for biosensor application. The usage of the protozoa poses several advantages- the absence of cell wall that can increase the sensitivity and having metabolic characteristics more similar to human cells. Amaro et al. [41] successfully created a transformed *T. thermophila* containing metallothionein promoters which could be turned on with the presence of heavy metals to express the linked luciferase gene.

Eukaryotic plant cells such *as D. carota* has been used in the study of whole cell biosensors [13]. *D. carota* cells suspension were utilized as the biological component, and the response of carotenoids in the cells after the exposure to heavy metals was detected with spectrometric approach. According to Wong and Choong [13], *D. carota* or carrot cell was chosen as the biological component because of the high carotenoids content found in the cell.

Besides, a genetically engineered yeast *Saccharomyces cerevisiae* has also been used as bioreceptor to detect heavy metals such as As, Fe, Pb, and Cd [42]. The engineering of this mammalian *CREBP-CRE* gene expression pathway together with green fluorescent protein reporter into yeast cells allowing the detection of heavy metals through the fluorescence emission.

6. ISSUES AND LIMITATIONS IN THE DEVELOPMENT OF WHOLE CELL-BASED BIOSENSORS

Turdean [43] reviewed that the usage of whole cell-based biosensors are limited by the understanding of the biochemistry involved, lack of genetic stability, short lifetime, require long contact period with analytes to produce significant responses, difficult to reverse the signal, the limitation of experimental condition, and the lack of selectivity over the analytes. In another review, Close et al. [44] highlighted that the challenges in the development of whole cell-based biosensors are to keep the whole cells viable through a long storage time and to immobilize the cells tight and close to the transducers.

The detection of bioavailable heavy metals is the most advantageous property of whole cell biosensors that enables the detection of pollutants which affect living cells [6, 33, 45]. But, the insensitivity to heavy metals which are not bioavailable to cells disabled these biosensors in measuring the total heavy metals in the environment. Besides, the amount of bioavailable pollutants that affect the microbial cells is different from human cells, which the discrepancies might cause miss-judgments on the toxicity effect to human body. Harms et al. [8] reported the difference of As bioavailability between bacterium and humans, which As found in the environment in the form of iron hydroxide colloids was not bioavailable to bacterium, but once consumed by human, the acidic stomach will release the heavy metal from iron hydroxides and bring toxicity effect to human cells.

Specificity is one important factor to be taken into consideration in the development of biosensors. Enzyme-based or antibody-based biosensors present high specificity on certain toxicants [46]. On the other hand, although cells are highly sensitive to the changes in surrounding environment, the wide variety of metabolic reactions in the cell towards heavy metals or pollutants in the environment reduce the specificity and selectivity of whole cell-based biosensors. Thus these biosensors are unable to quantitate the target analytes accurately due to high background noises or low signal to noise ratio [19]. Besides, the presence of a mixture of pollutants in environmental samples might reduce the performance of whole cell-based biosensors by acting either antagonistically or synergistically, due to cross reactivity [6,9,15,41].

In order to improve the sensitivity of the cell to pollutants, Wu et al. [19] came up with an idea known as a toggle sensor by inserting additional repressor gene to reduce the background fluorescence by non-specific inducers such as Isopropyl β-D-1-thiogalactopyranoside (IPTG) and increase the sensitivity of the cells towards Cd. The addition of nanobeads to whole cell biosensor development reported by Souiri et al. [47], had proven to increase the sensitivity of whole cell biosensor in heavy metals detection as well. In recent years, the utilization of recombinant microbes gains the popularity as the inserted genes can produce selected signals which are not available in naturally occurring cells [48-50]. The recombinant microbes can be used to enhance the sensitivity and specificity of the biosensors developed, however, as detailed review by Cases et al. [51], the extensive usage of recombinant cells should proceed by considering the effect of the transgenic organisms to the environment.

Cell density affects the performance of the biosensors in terms of signal transduction and sensitivity towards analytes. This can be seen ~~in~~ from *E. coli* based biosensor constructed by Sharma et al. [16], which high cell density reduced the light signal. Besides, Hillson et al. [17] reported that the *C. crescentus* based biosensor constructed for U detection would lead to false positive results due to the high cell density. Thus, the optimum cell density, which is varied by the types of cells and design of biosensors, has to be identified to ensure the best signal output from whole cell-based biosensors.

Immobilization of cells helps to increase the stability of the cells, reduce the risk of contamination, keeping the cells closer to the transducer, and increase the efficiency in receiving signals from reporter. However, appropriate immobilization methods is important to avoid the reduction in the stability of the cells either physically or chemically [25]. Some conventional materials such as agarose, agar, alginate, polyacrylamide, and chitosan are still highly preferred [52]. Recent report by Flickinger et al. [53] indicated that latex could be used to immobilize microbes for heavy metal detection. Besides, the usage of other materials e.g. silica matrix [54], nanocomposite film [55], immobilization onto cellulose membrane through simple filtration and poly(hydroxyethyl-methacrylate) (pHEMA) [22], and even the mixture of polystyrene-sulphonate-polyaniline [56] were documented.

7. CONCLUSION

The whole cell-based biosensors discussed in this review are some of the most popularly used biosensors for heavy metals detection. Although these biosensors have remarkable sensitivity and accuracy, the stability and storability of these bioanalytical tools are yet to be improved. Hence, further research and studies has to be carried out to further enhance the practicality of whole cell-based biosensors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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