Exploring the Potential of Whole Cell Biosensor: A Review in Environmental Applications

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***Abstract*—**Human activities have brought environmental pollution which biosensors can be effective tools to detect the presence of these pollutants. Whole cell biosensor is a category in biosensor design which cells are used as bioreporters, which can be coupled with different types of transducers. To date, many types of cells have been used in biosensor applications with the capability to detect vast range of pollutants, from heavy metals and pesticides, to biochemical oxygen demand. In this paper, several types of cells- bacteria, cyanobacteria, algae, plant cells, fungi, and protozoa, which were utilized in biosensors designed for environmental applications are reported. The possibility for further development of whole cell-based biosensors is review as well.

***Keywords*—**Biosensors, Whole cell, Environment pollutants

I. Introduction

HUMAN activities have resulted in the increase of environmental pollution. Biological tools such as biosensors offer wide-range of detection, hence making them flexible to be used as early warning system in the environment pollutants assessment. As defined by IUPAC, a biosensor is "a device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element, which is retained in direct spatial contact with a transducer element". Distinguished from bioanalytical system, a biosensor does not require additional processing steps such as reagent addition or sample purification. In addition to that, a biosensor should be distinguished from a single exposure probe [1]. Biosensors can be categorized on the basis of (i) presence of biological recognition element e.g. DNA, enzymes, antibodies and whole cell or (ii) the signal transduction method e.g. optical, electrical, mass-based and thermal [2].

The basic principle of whole cell biosensors is the immobilization of living cells or bacteria where these cells function as the molecular recognition elements that detect species of interest and translate them into functional information [3]. They have become an interest to provide preliminary degrees of analyte exposure on-site, also, providing sufficient precautionary measures and control developments at affected areas. The impact of biosensors varied and the application of biosensors in environmental monitoring continue to show advances various fields including mining, detection of heavy metals, biocides (pesticides, herbicides) and biochemical oxygen demand (BOD) [4, 5].

Different from other sensors available, whole cell biosensors have shown promising results in detecting the presence of various pollutants at the affected areas. Such reactions could be due to the interactions between the living cells in the biosensors with organic substances such as xenobiotics, heavy metals, changes in pH or radiation in water, soil and air. They continuously show potential in the aspects of environmental monitoring. The aim of this paper is to report the applications of bacteria, algae and other organisms in the development of biosensors and the possibility for further development.

II. Whole cell biosensor

A. Bacteria

Since the initiation of the first electrode-based biosensor [6], bacterial biosensors have become the highlight for the detection of pollutants in various fields with proven performance [7]. On this note, bacteria are more preferable as sensing elements due to their fast response, high growth rate and low cost. Bacterial biosensors depend on the promoter-reporter expression systems which compose of a transcription regulator and promoter or operator along with an open reading frame for proteins of measurable activity [8].

To date, most studies are directed on the detection of mercury (Hg), copper (Cu), uranium (U) and cadmium (Cd) [9-11], pathogens [12], toxins [13], but little research has been conducted for the mining industry. For example, the screening and analysis of gold would require the samples to be transported from the area of mining into a facility that analyses the gold and the processes require weeks to analyze samples. Recently, Zammit et al. [4] utilized bacteria for an on-site analysis for gold detection in the environment, where *golTSB* genes from *Salmonella enteric serova typhimurium* were selectively induced by Au (I/III)-complexes and

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integrated into *Escherichia coli* through DNA recombinant.

Although it is good news to be able to develop such simple, rapid and portable method to determine the location for new gold mines, it is an irony that the mining processes caused more contaminations in the ground water and it requires extensive studies for the clean-up processes. Such contaminants include Hg and arsenic (As). In this case, Ivask et al. [14] had developed fibre-optic bacterial biosensors for the analysis of bioavailability of Hg and As in soil and sediments. Such biosensors utilized recombinant-luminescent Hg and As-sensor bacteria onto optical fibres which were immobilized in alginate-based gel. The applications of fibre-optic biosensors had proven to be bioavailable to the bacteria for both Hg and As. In the same study, more than 20-fold of Hg and 4-fold of As were available to non-immobilized bacteria, which showed the importance of direct contact of the cells, which enhanced these metals in solid samples. Another example to show the development of whole-cell biosensors in measuring heavy metals in the environment is improved bacterial bioreporters by interfering with the natural homeostasis system of the host bacterium towards heavy metal. As current bioreporters lack of specificity and sensitivity, Hynninen [15] reported that a *Pseudomonas putida* KT-2440-based biosensor for zinc (Zn), Cd, and lead (Pb) detections were improved up to 45% through the disruption of four main efflux transporters, which caused the accumulation of metals within the cells.

In the case of biocides pollutions, *Pseudomonas fluorescens* HK44 was the first whole-cell bioreporter that was genetically modified with a bioluminescent (*luxCDABE*) gene that linked to a catabolic pathway of naphthalene degradation [16]. Since the development of the first whole-cell bioreporter in 1990, more reporter genes have been introduced to various host cells which including green fluorescence protein (GFP) and *lacZYA* [17, 18]. Table 1 describes the characteristics of commonly used reporter genes. For fast-acting biocides detection, microbiological techniques are commonly used to provide data on the rate and extent of kill for a range of biocides. However, these techniques have some limitations where the data obtained only described the initial rate of kill of fast-acting biocides in a short period of time. A study conducted by Robinson et al. [19] showed that the efficacy of recombinant *Escherichia coli* expressing *the Photorhabdus luminescens* lux operon as a whole-cell biosensor through the rapid analysis in detecting the fast-acting biocides within milliseconds of application. The assay also allows the confirmation of complete metabolic inhibition of the bioreporter.

So far, the applications of bacterial biosensors in environmental monitoring are broad. Therefore, it is not surprising that they also have been used in detecting the biochemical oxygen demand (BOD) in wastewater from industrial effluents. This is further supported through studies that had been performed by utilizing bacteria such as *Arthrobacter globiformis* and *Photobacterium phosphoreum* [20, 21]. However, employing bacteria for real-time detection of BOD are rare, perhaps due to technical issues that arise during the development of this type of biosensor or complicated metabolic pathways in certain type of bacteria which require further studies.

TABLE I
COMMONLY USED REPORTER GENES IN WHOLE-CELL BIOSENSORS AND THEIR CHARACTERISTICS

Reporter	Characteristics
lacZ	Chromogenic (X-gal, *o*-nitrophenyl galactoside) and chemiluminescent substrates are available. In *E. coli* host strains with the *lacZΔM15* mutation, only a small peptide representing the missing N-terminus, designated *lacZ′*α, is required.
luxAB	Blue bioluminescence in the presence of added substrate (a long-chain aldelhyde, usually decanal).
luxCDABE	As above; presence of *luxCDE* allows biosynthesis of the substrate so that it need not be added to the reaction.
Firefly or click-beetle luciferase	Bioluminescence in the presence of added substrate (D-luciferin). Quantum yield is higher than for bacterial luciferase, but the substrate is much more expensive. Luminescence is normally green, but color variants are now available.
Fluorescent proteins	Fluorescence when stimulated by ultraviolet or visible light. The original green fluorescent protein (GFP), still widely used, is stimulated best by ultraviolet; enhanced green fluorescent protein responds well to blue light, and numerous color variants are now available.

Source: Frech et al. [22]

B. Cyanobacteria

Cyanobacteria are considered as the oldest group of photosynthetic prokaryotes and are thought to be the first organisms to carry out oxygen-dependent photosynthesis; which led to their appearance as blue-green. Also known as blue-green algae, cyanobacteria contain chlorophylls which allow photosynthesis to occur. The pigments present in the chlorophylls convert the light absorbed into energy (photons) and a small amount of energy is released as fluorescence emission [23]. Currently, there are a few studies that utilize cyanobacteria for the development of whole-cell biosensor. Hence, this part of the discussion will be focusing solely on the usage of cyanobacteria in biosensors for the detection of heavy metals, pesticides, herbicides and biocides [24, 25].

A novel research conducted by Wong et al. [26] which employed a fluorometric whole-cell based biosensor using *Anabaena torulosa*, were found to be sensitive to Cu, Cd, Pb, and pesticides. The basis of this research is to develop a simple approach through entrapment Cyanobacteria *A. torulosa* onto a cellulose membrane through filtration. The membrane is soon dried and fixed into a cylindrical well, which was designed to attach to an optical probe which was connected to a fluorescence spectrometer with an optical fiber. The biosensor was tested with different combinations of toxicants and the results showed pre-dominantly antagonistic responses. These confirmed the constructed biosensor was suitable for qualitative and quantitative detections of heavy metals and pesticides. Besides *A. torulosa*, it is reported that another species of cyanobacteria, *Chlamydomonas reinhardtii* was employed for the development of a biosensor on the basis for multi-biomediators for the herbicide detection. The detection approach targets the activity of photosystem II (PSII); a multi-enzymatic chlorophyll-protein complex located in the thylakoid membrane that catalyzes the light-dependent photosynthesis [27]. Another study by Campanella et al. [28], reported that the coupling of cyanobacterium *Spirulina subalsa* to aperometric gas diffusion electrodes had allowed the monitoring of photosynthetic oxygen evolution and detecting any alterations in the system due to toxicants produced by the mankind in the environment.

Apart from the aspect of photosynthesis, another approach to quantitatively determine the presence of heavy metals is to conduct experiments based on the catalytic activity of the cells used. For example, Awasthi [29] employed *Anacystis nidulans* which contain alkaline phosphatase for the detection of nickel (Ni), Zn and Cd. In this study, the activity of the alkaline phosphatase was studied based on the p-nitrophenol formed in the culture suspension. Genetic engineering has allowed the modifications of various species of cyanobacteria and it includes the gene alteration with luciferase gene (*luc*) in detecting of various heavy metals and other forms of pollutants; therefore generating novel strains of cyanobacteria. Shao et al. [30] fused a freshwater cyanobacterium, *Synechocystis* sp. strain PCC6803 chromosome with luciferase gene (*luc* obtained from firefly *Photinus pyralis*) at the chromosome which generated a novel bioluminescent cyanobacterial strain.

Cyanobacteria are not as popularly used in biosensors compared to bacteria. It is believed that the future of cyanobacteria in whole-cell biosensors is enormous and there are plenty of space for future research and development.

C. Green Algae

Green microalgae are easy to culture and sensitive to various pollutants; therefore they are frequently employed for the screening of contaminated water [31]. The toxicity is measure through the monitoring of the inhibition of their photosynthetic activity; the estimation of the chlororophyll-α fluorescence of photosystem-II (PSII) or through the production of oxygen [32].

Algal biosensors are commonly used for the detection of heavy metals, biocides and BOD. For example *Chlorella vulgaris* was used in heavy metals detection because of their abundance in the nature and high metabolic activity towards various chemicals. A study conducted by a group researches had employed this species using conductometric biosensors consisting of gold planar inter-digitated electrodes and sol-gel algal membranes for the analysis of heavy metals (Cd, cobalt (Co), Ni, Pb, and Zn). These analytes were used to inhibit the alkaline phosphatase (AP) activity of *C. vulgaris* under optimum conditions. This biosensor was proven to be sensitive for the assessment of heavy metals in water [33]. Another study that employed the same approach and organism, was however has shown dual-functions where the biosensor detects both heavy metals and pesticides through disruptions two enzymatic activities (both alkaline phosphatase and esterase activities). Such biosensor was created by [34]. This analytical tool has the ability to screen for various pollutants that co-existing at the same location and can be used for real-time screening.

Apart from heavy metals detection and freshwater algae as the sensing element, there was a study that employed marine green algae *Ostreococcus tauri*– the smallest free-living eukaryotic cell as a new luminescent biosensor for the detection of anti-fouling biocides coastal waters. Different concentrations of anti-foulants were tested on four genetic constructs of *O. tauri* based on the genes involved in photosynthesis, cell cycle and circadian clock and were compared using a luminometer for the observation of the luminescence. The results showed that the luminescence cells showed high sensitivity towards the analyte. Cyclin-dependent kinase (CDKA) was fused with a reported gene (*luc*) to turn it into highly sensitive bioreporter. This biosensor owned the advantages over inhibition of cells growth as the test system is fully automated and able to provide a high-throughput laboratory approach for short-term tests of pollution in the environment [5]. Other than *O. tauri* and *C. vulgaris*, there are many species of microalgae that had been used for the screening of herbicides e.g. *Cictyosphaerium chlorelloids*, *Scenedesmus intermedius*, *Desmodesmus subspicatus*, *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii* [35-37].

Algal biosensors still have issues with specificity; hence the applications of algae-based sensors in the environment are still limited for screening purposes.

D. Fungi

Yeast and filamentous fungi occupying a wide range of niches in the environment and it would be reasonable to expect that they will collectively respond to a wide range of substrates. The utilization of yeast can provide various advantages over bacterial cells; (i) provide relevant and useful information to other eukaryotes; (ii) ease of cultivation and manipulation; and (iii) open to different transducer methodologies [38]. For example, naturally-occurring filamentous fungi such as *Armillaria mellea* and *Mycena citricolor* have shown their potentials in assessing toxicity of the environment through the production of luminescent light. They showed high response towards Cu, Zn, and pesticides. Another example of luminescent-producing yeast is the genetically modified *Saccharomyces cerevisiae*; whose gene was modified to express firefly luciferase. Such strain was developed so that any chemicals that interact with metabolism of the cells would display a quantitative decrease in the bioluminescence [39].

In the case of BOD analysis, there are several studies that employed fungi and yeast cells. The co-immobilization of *Trichosporon cutaneum* and *Bacillus licheniformis* developed by [40] provides dual reactions; where the immobilized microorganisms reacted towards glucose and glutamate respectively. The sensor developed allowed a rapid measurement of BOD. At pH range of 6.8 to 7.2, this microbial sensor unfortunately showed low sensitivity. The Japanese Industrial Standard also used the same fungi as the immobilized biocatalyst in a surface photo-voltage (SPV) device. *T. cutaneum* was integrated between the membrane filters in SPV (sensitive to surface pH). The pH measured was characterized by comparison with the 5 day BOD test (BOD5) and the sensors used in BOD measurement (BODs). The results were good enough to applied for real waste water BOD measurement [41]. Back in 1996, there had already been a study of BOD sensor with broad functions, including analyzing all types of organic contaminants using *Torulopsis candida* as an alternative to *T. cutaneum* [42].

E. Plant Cell

Wong & Choong [43] reported the usage of *Daucus carota* cell suspension as the biological sensing element. The respond of the caretonoid compounds after exposure to heavy metals was detected by using an optical transducer (a spectrophotometer). The reason for choosing carrot cells was because of the high amount of caretanoids present in the cells, which would elevate when the concentration of heavy metals increased. It was due to the counteract action of the caretanoids towards the oxidative stress resulted from the heavy metals.

F. Protozoa

Tetrahymena thermophila was the first ever ciliated protozoan biosensor used for the detection of heavy metals. This organism lacks of cell wall, hence making it highly sensitive towards any chemicals and also it has similar metabolic pathways as in human cells in relative to yeast or bacterial cells. Hence, it can be used as a reference for the study of impact of heavy metals towards human cells [44]. A study done by Amaro et al. [44] showed the action of recombinant *T. thermophila* whose promoters (MTT 1 and MTT5) were linked to eukaryotic luciferase gene. The amount of heavy metals present at the affected area was quantitatively measured with the luminescent light generated by these transformed cells.

III. Conclusion

Whole cell biosensors have a huge future ahead, especially in the environmental applications. With the pollution increasing from time to time, analytical tools for rapid, simple, accurate and on-site tools are highly needed. Conventional methods may be the better for analysis of some contaminants but require funds and experts, which may limit the application of these approaches in broader field. Thus, further research and development is required to enhance the ability of the whole-cell biosensors to perform at their maximum potential.

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