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The Effectiveness of Genetically Modified Microbes in Biosensors for Environmental Applications

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Abstract: Environmental pollution is worsening due to human activities. Biosensors are an alternative analytical tool to eliminate pollutants in environment. Through the advancement of recombinant technology, genetically modified microbes are now available to be integrated into a biosensor design. The microbes have the ability to produce signals which are not available in the wild type counterpart with certain modified characteristics. In this paper, the application of the genetically modified microbes has been discussed. The performance of the genetically modified microbes in biosensors has been focused and comparison has been done with the wild type counterparts. The future development of biosensors with recombinant microbes require more research in the areas of sensitivity, specificity, and the ability of biosensors to operate under stressed environment are discussed as well.

I.Introduction

Biosensors are commonly defined as a combination of biological component, transducer, and electronic reader. Biosensor is an analytical device used to observe changes in biological reactions into an electrical signal output [1].The principle component of a biosensor is its bio-recognition component, and that component can unequivocally collaborate with its target analyte. Followed by the installation of the transducer, it creates responses that can be examined and deciphered through information produced by electronic reader [2]. The collaboration of bio-recognition component with its target analyte is capable to capture the changes in the pH, temperature, light discharge, electron exchange and more [3].

Whole cells, especially microbes have been widely used in the development of biosensors in environmental applications. The whole cells have been regularly proposed as bio-recognition components as a part of numerous applications is the use of whole cell organism, especially the microbial cells [4-7]. As of late 80's, recombinant microorganisms have been highly preferred for its role in two important bearings in the improvement of biosensor toxicity bioassays, which known as "lights off" and "lights on" tests. The concept is an extension of the generally used microbial bioassay, based upon estimation of the reduction in light illumination. For example, the most commonly used bacterium for this purpose is the wild-type luminescent bacterium *Vibrio fischeri* [8]. Various types of microorganisms were also adjusted to constitutively luminescent and accordingly to serve as potentially reliable markers for toxicity bioassay. As an example of recombinant bacteria, *Escherichia coli* Hb101 which harbours luxCDABE of *V. fischeri* was immobilized in polyvinyl beads [8]. Another bacteria which is the *Pseudomonas fluorescens* also used the same methodology. Furthermore, the cyanobacterium *Synechocystis* Pcc6803 with the luc gene from the firefly *Photinus pyralis*, also explored in this methodology [8]. Apart from that, various other microorganisms were modified to give the best in interest to develop a higher end luminesce biosensor [8].

Many human activities caused pollution from point sources (e.g. modern industrial effluents, refining wastes) and non-point sources such as the solvent salts, insect sprays and pesticides [9]. These tainting substances have different levels of impact on the environment and life forms. Past studies confirmed that contamination has severely lowered the quality of arable lands [10] while deteriorating the quality of water [9].

Highly recognized devastating heavy metals that contaminates environment includes As, Cu, Cd, Pb, Cr, Ni, Hg and Zn [9]. These inorganic pollutants are released into environment through industrial, agricultural, and the disposal of domestic wastes. Meanwhile organic pollutants are the compound that mainly consists of hydrogen and carbon atoms. These organic pollutants include compounds originate from pharmaceuticals, pesticides, food additives and industrial byproducts [11]. Wastewater from various industries, livestock and agriculture are the main resources of these organic pollutants [11]. These pollutants affect the ecosystem and have numerous toxic effects to human well-being. An incident occurred in 1968, where more than one thousand people ate polychlorinated biphenyls (PCBs)-contaminated rice oil in Yusho, Japan. Ten years later in 1979, a similar incident occurred in Yucheng, Taiwan [13]. Li et al. [14] reported that the affected people showed an increased mortality patterns 30 years after the exposure. Organic pollutants such as PCBs are persistent in the environment where it require few centuries for degradation.

In this review paper, the focus is on the effective applications of genetically modified microorganisms in optical biosensor for environmental pollutants detection. Apart from discussing successful applications of genetically modified microorganism biosensor, comparisons were also made with wild-type microorganism biosensor on based on different variables.

II.Genetically modified microbial biosensor and the wild type microbial biosensor for metal pollutant detection

The development of genetically modified microbes for biosensor applications has been widely reported. For example, *E. coli* XL1-Blue with *zra*P and *cus*C promoters fused with *rfp* and *gfp* reporter genes were able to detect the copper and zinc down to of 5.10 mg/L (32 μM) and 2.59 mg/L (19 μM) respectively. This is below the recommended toxic levels for zinc and copper provided by the guidelines in the United States Environmental Protection Agency which is 5 mg/L for zinc [15]. *E. coli* MC1061 with merR/Pmer gene and *luxCDABE* reporter gene were able to detect mercury (II) at the level of 1 x 10^{-3} mg/L [16] and 3 × 10^{-5} mg/L [17]. These lowest limit detections were lower than permissible level set by United States Environmental Protection Agency (EPA) in which by default was 0.002 mg/L. Ivask, Rõlova and Kahru [17] utilized genetically modified *E. coli* MC1061 with combination of different regulatory genes such as pbrR/pbrA and cadR/cadA gene for mercury (II) detection, but with lower sensitivity compared to the biosensor developed using *E. coli* MC1061. Thus, the construction of an effective recombinant biosensor requires an appropriate regulatory gene in order to optimize the sensitivity towards specific metal.

III.Metal toxicity tolerance of recombinant microbial biosensor

Ivask, Rõlova and Kahru [20] reported the tolerance level of the recombinant microbes was higher in the recombinant bacterial biosensor than the wild type counterpart. In their study, the transformation of promoter *cad*A and *cad*R into *Staphylococcus aureus* RN4220 strain showed that, 2.75 mg/L of cadmium chloride was required to induce the toxicity. Whereas, only 0.2 mg/L of cadmium chloride was strong enough to induce toxicity for the wild-type *S. aureus* RN4220 strain. In another toxicity tolerance study done by Stoyanov, Magnani, and Solioz [70], *E.coli* *cop*A-knockout strain DW3110 was used to compare with its wild type strain. It was reported that an eight-fold higher concentration of argentum and a fifthteen-fold higher concentration of copper were required to induce maximum toxicity response in the recombinant strain compared to the wild type.

In certain cases, the genetically modified microbial biosensors were unable to detect a very low concentration of metal pollutants [18-19]. The lowest limit detection of copper (II) and cadmium (II) metals using recombinant *E.coli* with *cop*R and *cop*A upstream *lacZ* gene was only able to detect at concentration of 15.961 mg/L [20]. Comparatively in another research, the wild-type cyanobacteria *Anabaena torulosa* was able to detect these metals to the extent as low as 0.0022 mg/L [21] and 2.7 × 10^{-5} mg/L [22] respectively. Higher tolerance to the toxicity means lower sensitivity, but on the other hand, these specific genes could be potentially used in the detection of higher concentration of silver and copper toxic in the environment.

IV.Specificity of the genetically modified microbial biosensor towards metal pollutants

Genetically modified microbial biosensors were found to be highly specific to a certain group of metals. Tibazarwa et al. [23] constructed a genetically modified *Ralstonia eutropha* strain AE2515 using *cnr*YXH regulatory gene upstream of *luxCDABE* reporter gene, specifically for nickel and cobalt detections. The biosensor did not showed any response to zinc, chromium (III and V), manganese, cadmium (II) and copper (II) ions in the conformation test. A green fluorescent protein (GFP)-based bacterial biosensor *E.coli* DH5a was constructed by Liao et al [24], gave a positive response only towards Cd (II), and without interference from Pb(II), Sb(III) Zn(II) ions. A yellow fluorescence-based *E.coli* DH5α with *ars*R gene was constructed by Sharma, Asad and Ali [25] for arsenite detection and *P. fluorescens OS8* which was transformed with *cueR* and *copA* gene for Cu (II) ion detection [17].

The specificity of genetically modified microbial biosensors may be caused by the production of several proteins encoded by different metal-response genes. These genes may come from a same family, for example metal binding regions of *ars*R protein (which is specific for arsenic) are assumed to be conserved and the regulation protein of the cadA-operon and CadC protein (which specific for cadmium), are found similar tothe member of *ars*R protein family [26]. Another factor that contributes to the specificity is the specific enzyme produced by genetically modified microbes in response to the presence of specific metal ions, for example *E. coli* strain Bl21 that carries *ars*R gene and *gfp* gene is capable to produce arsenate reductase, which the production of the enzyme is triggered by the presence of arsenate [27].

V.Sensitivity of genetically modified microbial biosensor and wild type microbial biosensor

Several evidence confirmed the sensitivity of genetically modified microbial biosensors towards organic pollutants. Jia et al. [34] had successfully constructed *E.coli* TV1061 strain with *grp*E promoter fused with *luxCDABE* gene. The outcome of the study provided a detection of atrazine in drinking water as low as 1 x 10^{-14} mg/L, where the standard set by the United State Environment Protection Agency is 3 x 10^{-3} mg/L [35]. It was much more sensitive compared to wild type *Chlorella vulgaris* used by Naessens, Leclerc & Tran-Minh [36], which able to detect atrazine to the level of 2.16 ×10^{-5} mg/L. In another research using wild type *Chlamydomonas reinhardtii* [37], the lowest limit of detection of atrazine recorded was 2.2 x 10^{-3} mg/L.

A cyanobacteria recombinant biosensor using *Synechocystis sp*. strain PCC6803 marked with the firefly luciferase gene *luc*, was reported to be more sensitive than the wild type green alga *Selenastrum capricornutum* for the detection of glyphosate. *Synechocystis sp*. strain PCC6803 gave shorter response time compared to *S. capricornutum* which has taken 4 days [38]. Even so, several biosensors utilizing wild type cells showed a good sensitivity in terms of the effective concentration. The reduction of growth by 50% (EC_{50}) were reported with the wild type green algae *Dunaliella tertiolecta* which was 5.9 µg/L. *Synechococcus* sp. strain PCC 543 and luc-marked *Synechocystis sp*. strain PCC6803 were both reported 5.50 x 10^{-1} µg/L and 2.97 x 10^{4} µg/L of EC_{50} respectively, with exposure times of 72 hours and 96 hours respectively.

The level of toluene in drinking water set by EPA and World Health Organization is not exceeding 0.70 mg/L [39]. Two interesting studies were conducted using recombinant microbial biosensor of *E.coli* DH5a harbouring plasmid pGLPX1[33] and *E. coli* strain TG1 harbouring plasmid pBS(Kan)TOM [40] showed the biosensors developed were able to detect toluene as low as 9.20 x 10^{-2} mg/L and 2.77 x 10^{-2} mg/L respectively. However, certain recombinant microbial biosensors could not give the same successful detection rate, for example two biosensors developed using *Pseudomonas putida* mt-2 KG1206 strains [41] and *E. coli* DH5a cells harbouring the pTOLLUX plasmid [48] could only detect toluene at 9.21 mg/L and 691.05 mg/L respectively.

VI.Reliability and accuracy of the recombinant microbial biosensor on pollutants

The reliability and accuracy of genetically modified microbial biosensor for organic pollutant detection was validated with the conventional chemical analysis, such as high liquid performance chromatography. Many biosensors yielded highly similar to the outcome from the chemical analysis [43-45]. Shin [45] reported that the concentrations of phenolic compounds in hospital wastes tested with immobilized *E. coli* cells harbouring *lacZ* and CapR gene were similar to the results obtained from the chemical analysis. A few others reported the same trend of analysis even though certain chemical impurities and solvent extraction were present in the results [46-47].

The regulatory protein that expressed by pollutant responsive gene could often response well to a certain targeted organic pollutants what share similar functional group, which the pollutants fit into the effector-binding sites. For example, the regulatory proteins that reacted with toluene, were found to react with benzene, xylene, phenol and several other organic compounds as well [40-44, 47-48]. Thus, majority of research on genetically modified microbial biosensors showed the detection of a group of organic compounds rather than a specific. Some limitations were reported as well. In a few biosensors, sensitivity over the compounds with similar functional group varied significantly, and as these recombinant microbes were sensitive to the organic compounds with similar functional group, the biosensors developed using these microbes might not suitable for broad spectrum analysis [47-48].

Genetically modified microbial biosensors were tested for its ability of detecting metal pollutants in the sample taken from the soil and water [15, 24, 25, 31, 33, and 49]. The recombinant biosensors were able to differentiate the polluted sample from the non-polluted sample with high precision in quantitative analysis [15, 31, 33, and 49]. *Pseudomonas putida* X4 carries *czcR3* promoter and *egfp* reporter gene for zinc metal detection in water sample from red soil, brown soil, and cinnamon soil showed a good correlation as well [31]. However, there was discrepancy between atomic absorption spectrophotometry analysis for *P. putida* X4 in detecting zinc in black soil and *Synechocystis* sp. PCC 6803 were unable to produce the luminescence signal upon incubation with the undiluted acetic acid from the contaminated soil

VII.The reaction rate of recombinant and wild type biosensor

The time required for most of the genetically modified microbial biosensors to produce a response is different, depending on the design of the recombinant cells, the biosensors design, and the pollutants. Several wild type microbial biosensors have been reported to consume only few minutes for a measurable responses [36, 50-51]. The exposure time recorded by some wild type microbes is much shorter than the recombinant one. This can be explained as most of the wild type microbial biosensors detect the target pollutants using the photosynthetic pigments or enzymes which already present *in vivo* naturally. Comparatively for the genetically modified microbial biosensors, especially for the luminescence and fluorescence based biosensors, the target pollutants have to be accumulated up to a certain level to induce the activation of the depressed reporter gene from the repressor protein and to speed up the slow formation of reporter proteins [48, 52]. However, a great advantage for the recombinant proteins, e.g. green fluorescence protein is the stability of the protein, where the detection is reported even after the cell death [53] and this characteristic is

important for the detection of highly toxic environmental pollutants [48].

VIII. Conclusion

The effective applications of genetically modified microorganisms in optical biosensor for environmental pollutants detection is successfully discussed in the paper. Applications of genetically modified microorganism biosensor and its comparisons with wild-type microorganism biosensor based on different variables also intensively been discussed. In sum, it was justified that the genetically modified microorganism based biosensor have contributed a lot more advantages compared to its wild type microorganism biosensor in so many analytical and research aspects.

References

[1] M. Datta, "Development of biosensor for heavy metal detection,", unpublished, http://dspace.thapar.edu:8080/dspace/handle/10266/2183, March 2013

[2] Y. Xu, "A Generic Smell Generating Enzymatic Biosensor," Unpublished thesis, 2011, Retrieved from http://digitalcommons.mcmaster.ca/opendissertations/6165/

[3] L. S. Cock, A. Arenas, and A. Aponte, "Use of enzymatic biosensors as quality indices: A synopsis of present and future trends in the food industry," Chilean Journal of Agricultural, vol 69, pp. 270–280. June 2009, Retrieved from http://www.scielo.cl/pdf/chiljar/v69n2/at17.pdf

[4] H. Fujimoto, M. Wakabayashi, H. Yamashiro, I. Maeda, K. Isoda, M. Kondoh, M. Kawase & H. Miyasaka and K. Yagi, "Whole-cell arsenite biosensor using photosynthetic bacterium *Rhodovulum sulfidophilum* as an arsenite biosensor," Applied Microbiology and Biotechnology, vol 73, pp. 332–338, May 2006

[5] L. H. Hansen, and S. J. Sørensen, "The Use of Whole-Cell Biosensors to Detect and Quantify Compounds or Conditions Affecting Biological Systems," Microbial Ecology, vol 42, no. 4, pp. 483-494, December 2001

[6] L. S. Wong, S. Surif, and Y. H. Lee, "Toxicity biosensor for the evaluation of cadmium toxicity based on photosynthetic behavior of cyanobacteria Anabaena torulosa," Asian Journal of Biochemistry, vol 3, no. 3, pp. 162-168, 2008

[7] C. Vedrine, J. C. Leclerc, C. Durrieu, and C. Tran-Minh, "Optical whole-cell biosensor using *Chlorella vulgaris* designed for monitoring herbicides," Biosensors and Bioelectronics, vol 18, pp. 457 – 463, 2003

[8] S. Belkin, "Genetically engineered microorganisms for pollution," in Soil and Water Pollution Monitoring, Protection and Remediation, Springer Netherlands, pp.147–160, 2006

[9] M. I. Lone, Z. He, P. J. Stoffella, and X. Yang, "Phytoremediation of heavy metal polluted soils and water: progresses and perspectives," Journal of Zhejiang University. Science. B, vol 9, no. 3 pp. 210–220, March 2008

[10] X. Liu, Q. Wu, and M. K. Banks, "Effect of simultaneous establishment of Sedum alfredii and Zea mays on heavy metal accumulation in plants," International Journal of Phytoremediation, vol 7, pp. 43–53, 2005

[11] D. J. Lapworth, N. Baranb, M. E. Stuart, and R. S. Ward, "Emerging organic contaminants in groundwater: A review of sources, fate and occurrence," Environmental Pollution, vol 163, pp. 287-303, April 2012

[12] A. Pal, K. Y. H. Gin, A. Y. H. Lin, and M. Reinhard, "Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects," Science of the Total Environment, vol 408, no. 24, pp. 6062-6069, December 2010

[13] D. Haffner, and A. Schecter, "Persistent Organic Pollutants (POPs): A Primer for Practicing Clinicians." Current Environmental Health Reports, vol 1, pp. 123–131, June 2014

[14] M. C. Li, P. C. Tsai, P. C. Chen, C. J. Hsieh, Y. L. L. Guo, and W. J. Rogan, "Mortality after exposure to polychlorinated biphenyls and dibenzofurans: 30 years after the ''Yucheng Accident,'''' Environmental Research, vol 120, pp. 71-75, January 2013

[15] S. Ravikumar, I. Ganesh, I. K. Yoo, and S. H. Hong, "Construction of a bacterial biosensor for zinc and copper and its application to the development of multifunctional heavy metal adsorption bacteria," Process Biochemistry, vol 47, no. 5, pp. 758-765, May 2012

[16] A. Ivask, T. Green, B. Polyak, A. Mor, A. Kahru, M. Virta and R. Marks, "Fibre-optic bacterial biosensors and their application for the analysis of bioavailable Hg and As in soils and sediments from Aznalcollar mining area in Spain", Biosensors and Bioelectronics, vol 22, pp. 1396–1402, 2007

[17] A. Ivask, T. Rõlova, and A. Kahru, "A suite of recombinant luminescent bacterial strains for the quantification of bioavailable heavy metals and toxicity testing," BMC Biotechnology, vol 9, no. 41, pp. 1-15, May 2009

[18] K.Yagi, "Applications of whole-cell bacterial sensors in biotechnology and environmental science", Appllied Microbiology and Biotechnology, vol 73, no. 6, pp. 1251-1258, 2006

[19] H. J. Shin, "Genetically engineered microbial biosensors for in situ monitoring of environmental pollution," Applied Microbiology and Biotechnology, vol 89, no. 4, pp. 867–877, February 2011

[20] S. P. Ng, E. A. Palombo, and M. Bhave, "Identification of a copper-responsive promoter and development of a copper biosensor in the soil bacterium Achromobacter sp. AO22," World Journal of Microbiology Biotechnology, vol 28, no. 5, pp. 2221–2228 , 2010

[21] L. S. Wong, Y. H. Lee and S. Surif, "The fluorometric response of cyanobateria to short exposure of heavy metal," Advances in Environmental Biology, vol 6, no. 1, pp. 103-108, 2012

[22] L. S. Wong, Y. H. Lee and S. Surif, "Whole cell biosensor using Anabaena torulosa with optical transduction for environmental toxicity evaluation," Journal of Sensors, 2013

[23] C. Tibazarwa, P. Corbisier, M. Mench, A. Bossus, P. Solda, M. Mergeay, L. Wyns, and D. van der Lelie, "A microbial biosensor to predict bioavailable nickel in soil and its transfer to plants, " Environmental pollution, vol 113, no. 1, pp. 19-26, 2001

[24] V. H. C. Liao, M. T. Chien, Y. Y. Tseng, and K. L. Ou, "Assessment of heavy metal bioavailability in contaminated sediments and soils using green fluorescent protein-based bacterial biosensors," Environmental Pollution, vol 142, pp. 17-23, July 2006

[25] P. Sharma, S. Asad, and A. Ali, "Bioluminescent bioreporter for assessment of arsenic contamination in water samples of India," Journal of Bioscience, vol 38, no. 2, pp. 251–258, June 2013

[26] S. Tauriainen, M. Karp, W. Chang and M. Virta, "Luminescent bacterial sensor for cadmium and lead," Biosensors and Bioelectronics, vol 13 pp. 931–938, 1998

[27] M. Daneshpour, N. Shabab, A. Roointan , A. Rahmani,G. A. Chehardoli and M. Saidijam, "Designing a bacterial biosensor for arsenic detection in water solutions", International journal of medical investigation, vol 3, no. 3, pp. 91-100, October 2014

[28] Y. Xu, "A Generic Smell Generating Enzymatic Biosensor," Unpublished thesis, 2011, Retrieved from http://digitalcommons.mcmaster.ca/opendissertations/6165/

[29] S. P. Ng, E. A. Palombo, and M. Bhave, "Identification of a copper-responsive promoter and development of a copper biosensor in the soil bacterium Achromobacter sp. AO22," World Journal of Microbiology Biotechnology, vol 28, no. 5, pp. 2221–2228 , 2010

[30] P. Gireesh-Babu and A. Chaudhari, "Development of a broad-spectrum fluorescent heavy metal bacterial biosensor," Mol. Biol. Rep., vol 39, pp. 11225–11229, October 2012

[31] P. L. Liu, Q. Y. Huang, and W. L. Chen, "Construction and application of a zinc-specific biosensor for assessing the immobilization and bioavailability of zinc in different soils," Environmental Pollution, vol 164, pp. 66-72, May 2012

[32] C. R. Arias-Barreiro, K. Okazaki, A. Koutsaftis, S. H. Inayat-Hussain, A. Tani, M. Katsuhara, K. Kimbara & I. C. Mori, "A Bacterial Biosensor for Oxidative Stress Using the Constitutively Expressed

Redox-Sensitive Protein roGFP2," Sensors, vol. 10, pp. 6290-6306, June 2010

[33] F. Behzadian, H. Barjeste, S. Hosseinkhani and A. R. Zarei, "Construction and characterization of Escherichia coli whole-cell biosensors for toluene and related compounds," Curr Microbiol, vol 62, pp. 690–696, September 2011

[34] K. Jia, E. Eltzov, T. Toury, R. S. Marks, and R. E. Ionescu, "A lower limit of detection for atrazine was obtained using bioluminescent reporter bacteria via a lower incubation temperature,", Ecotoxicology and Environmental Safety, vol 84, pp. 221–226, October 2012

[35] United States Environmental Protection Agency, Basic Information about Atrazine in Drinking Water, http://water.epa.gov/drink/contaminants/basicinformation/atrazine.cfm, 2012

[36] M. Naessens, J. C. Leclerc, and C. Tran-Minh, "Fiber optic biosensor using Chlorella vulgaris for determination of toxic compounds," Ecotoxicology and Environmental Safety, vol 46, no. 2, pp. 181-185, June 2000

[37] Y. Ferro, M. Perullini, M. Jobbagy, S. A. Bilmes, and C. Durrieu, "Development of a biosensor for environmental monitoring based on microalgae immobilized in silica hydrogels," Sensors, vol 12, pp. 16879-16891, December 2012

[38] C. Y. Shao, C. J. Howe, A. J. R. Porter, and L. A. Glover, "Novel cyanobacterial biosensor for detection of herbicides," Applied and Environmental Microbiology, vol 68, no. 10, pp. 5026–5033, October 2002

[39] WHO, Guidelines for drinking water quality. 3rd ed, incorporating the first and second addenda. World Health Organization, Geneva, Switzerland, 2008

[40] Z. Zhong, M. Fritzschea, S. B. Pieper, T. K. Wood, K. L. Lear, D. S. Dandy and K. F. Reardon, "Fiber optic monooxygenase biosensor for toluene concentration measurement in aqueous samples," Biosensors and Bioelectronics, vol 26, 2407–2412, 2011

[41] I. C. Kong, H. Suh, Z. Yang, and R. S. Burlage, "A bioluminescent reporter strain utilizing the lower pathway promoter (Pm) of the xyl operon of Pseudomonas: optimization of a bioassay for m-toluate," Advances in Environmental Research, vol 8, no. 3-4, 647–654, March 2004

[42] Y. F. Li, F. Y. Li, C. L. Ho, and V. H. C. Liao, "Construction and comparison of fluorescence and bioluminescence bacterial biosensors for the detection of bioavailable toluene and related compounds," Environmental Pollution, vol 152, no. 1, pp. 123-129, March 2008

[43] M. Zeinoddini, K. Khajeh, F. Behzadian, S. Hosseinkhani, A. R. Saeedinia, and H. Barjesteh, "Design and characterization of an aequorin-based bacterial biosensor for detection of toluene and related compounds," Photochemistry and Photobiology, vol 86, pp. 1071–1075, September 2010

[44] S. Gupta, M. Saxena, N. Saini, Mahmooduzzafar , R. Kumar and A. Kumar, "An effective strategy for a whole-cell biosensor based on putative effector interaction site of the regulatory DmpR protein," PLOS ONE, vol 7, no. 8, pp. 1-11, August 2012

[45] H. J. Shin, "Agarose-gel-immobilized recombinant bacterial biosensors for simple and disposable on-site detection of phenolic compounds," Applied Microbiology and Biotechnology, vol 93, no. 5, pp. 1895–1904, March 2012

[46] S. M. Park, H. H. Park, W. K. Lim, and H. J. Shin, "A new variant activator involved in the degradation of phenolic compounds from a strain of *Pseudomonas putida*," Journal of Biotechnology, vol 103, no. 3, pp. 227 – 236, August 2003

[47] J. Trögl, S. Ripp, G. Kuncova, G. S. Sayler, A. Churava, P. Parik, K. Demnerova, J. Halova, and L. Kubicova, "Selectivity of whole cell optical biosensor with immobilized bioreporter *Pseudomonas fluorescens* HK44," Sensors and Actuators B, vol 107, pp. 98–103, 2005.

[48] A. Leedjarv, A. Ivask, M. Virta, and A. Kahru, "Analysis of bioavailable phenols from natural samples by recombinant luminescent bacterial sensors," Chemosphere, vol 64, no. 11, pp. 1910–1919, September 2006

[49] R. Branco, A. Cristovao, and P. V. Morais, "Highly Sensitive, Highly Specific Whole-Cell Bioreporters for the Detection of Chromate in Environmental Samples", PLOS ONE, vol 8, no. 1, Jan 2011

[50] E. Pena-Vázquez, E. Maneiro, C. Pérez-Conde, M. C. Moreno-Bondi, and E. Costas, "Microalgae fiber optic biosensors for herbicide monitoring using sol–gel technology," Biosensors and Bioelectronics, vol 24, no. 12, pp. 3538–3543, August 2009

[51] N. Das, and K. F. Reardon, "Fiber-optic biosensor for the detection of atrazine: characterization and continuous measurements," Analytical Letters, vol 45, pp. 251-261, Feb 2012

[52] K. Hakkila, M. Maksimow, M. Karp, and M. Virta, "Reporter Genes lucFF, luxCDABE, gfp, and dsred have different characteristics in whole-cell bacterial sensors," Analytical Biochemistry, vol 301, pp. 235–242, February 2002

[53] E. Sagi, N. Hever, R. Rosen, A. J. Bartolome, J. R. Premkumar, R. Ulber, O. Lev, N. Scheper, and S. Belkin, "Fluorescence and bioluminescence reporter functions in genetically modified bacterial sensor strains," Sensors and Actuators B, vol 90, no. 1-3, pp. 2–8, April 2003