Development of an Optical Whole Cell Microalgal-Cyanobacterial Array Biosensor to Assess Bioavailability of Selected Heavy Metal Pollutants in Aquatic Systems

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ABSTRACT

Pollution of aquatic ecosystems by toxic pollutants is a serious issue, globally as well as in Sri Lanka. One of the most predominant pollutants that pose a major threat in water pollution is the pollution by toxic heavy metals. Addition of heavy metals to ecosystems is rapidly increasing due to anthropogenic sources. The non-biodegradable nature of heavy metals causes high risk to organisms than other pollutants. Therefore, it is essential to monitor the heavy metal pollution in aquatic environments. In this research, biosensor approach was used as a novel method to detect bioavailable toxic heavy metals in aquatic systems. Changes of fluorescence in microalgae and cyanobacteria in the presence of heavy metals were used to develop a biosensor that can be used in environmental monitoring.

A total of six strains (three genera) of microalgae and four strains (four genera) of cyanobacteria were isolated from different local aquatic environments and the microplate toxicity test for heavy metals was carried out for each of the microorganisms to select sensitive strains of microalgae and cyanobacteria suitable for the development of a general non-specific biosensor. The relative fluorescence (%) patterns based on observed changes in chlorophyll a fluorescence in isolated microorganisms in the presence of heavy metals Cr^{6+}, Cd^{2+} & Zn^{2+} indicated that the microalgae *Mesotaenium* sp. 2 [EC_{50} 51.9 (±2.7) μg/L for Cr^{6+}] and *Mesotaenium* sp. 3 [EC_{50} 57.6 (±3.0) μg/L for Cd^{2+}; EC_{50} 1362 (±111) μg/L for Zn^{2+}] and the cyanobacterium *Synechococcus* sp. [EC_{50} 60.9 (±2.9) μg/L, 72.7 (±3.6) μg/L, 1795 (±155) μg/L for Cr^{6+}, Cd^{2+} and Zn^{2+} respectively] were the most sensitive and therefore the most suitable microorganisms for the development of an array biosensor consisting of both eukaryotic microalgae and prokaryotic cyanobacteria. Selected three microorganisms were immobilized in a 96-well microplate by employing sol-gel method using silica as the entrapment medium. Optimum operational conditions such as metal ion exposure time, storage stability, pH of the medium and multiple metal effect were tested for the biosensor. Validation of the biosensor was carried out using environmental samples taken from four effluent water discharge sites polluted with heavy metals. In the optimization of the biosensor, it was observed that 10 minutes exposure time yielded optimum fluorescence values, while metal toxicity increased with decreasing pH resulting in low relative fluorescence (%) and decreased with increasing pH resulting in higher relative fluorescence (%). The optimum storage time of the two biosensor algal cultures was four weeks while the optimum storage time for the cyanobacterial culture was eight weeks, at 4 ^{0}C storage conditions. The metal mixtures showed less effect on the inhibition of relative fluorescence (%) of microalgal/cyanobacterial cultures displaying an antagonistic behavior among the metals tested.

In Sri Lanka no scientific reports are available in developing whole cell microbial biosensors for environmental monitoring, and risk assessment particularly for heavy metals. Therefore biosensor array developed in this study would be useful for screening of effluents after the waste treatment for the bioavailable metals before discharge into water bodies, routine environmental monitoring practices, etc. with respect to the metals studied. Further studies using other heavy metals and organic contaminants are warranted for further characterization of the biosensor in order to expand its use for aquatic pollution monitoring.

Key words: Microalgae, cyanobacteria, heavy metals, biosensor, fluorescence