FY 2010 RESEARCH PROBLEM STATEMENT

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TITLE (*more info*)

Development of Gene Based Biosensors for Monitoring of Highway Stormwater Runoff

PROBLEM (Description of need) **(*more info*)**

Highway stormwater runoff is an important non-point source of many contaminants present in aquatic environmental systems. These contaminants, many of which are toxic to aquatic organisms at traces levels, include aromatic and chlorinated hydrocarbons, heavy metals, and pesticides. The presence and concentration of these pollutants are widely variable both temporally and geographically. In addition, due to the large number of potential pollutants and the correspondingly large number of analytical procedures necessary to completely characterize stormwater, monitoring efforts are complicated, time-consuming and costly. Furthermore, results from the analyses are often not known for hours to days after the storm event. ODOT's stormwater management and monitoring efforts would be improved with the development of a tool for rapidly screening stormwater for the presence of various toxicants.

PROPOSED RESEARCH, DEVELOPMENT OR TECHNOLOGY TRANSFER ACTIVITY (*more info*)

The proposed research will make advances towards the practical application of real-time gene-based biosensors for monitoring stormwater runoff. Through current NSF funding a team of microbiologists, biochemists and engineers at Oregon State University have been evaluating gene expression of the bacteria Nitrosomonas europea upon exposure to low levels of contaminants in water. Sentinel genes have been identified that provide unique signals upon exposure to heavy metals, aromatic and chlorinated hydrocarbons. In the NSF funded work, upregulation of gene expression was observed at concentrations of zinc (Zn^{+2}) and cadimum (Cd^{+2}) as low as 1 uM. Organic chemcials, for example, phenol, creosol, and chloroform, showed upregulation of different genes, upon exposure to concentrations in the range of 5 uM. In the proposed work, the team would evaluate how these genes would respond to contamination in samples of stormwater runoff. Nitrosomonas europea would be used as the biosensor. Three different approaches would be evaluated. In the first approach, all the sentinel genes that have been identified thusfar for the chemicals studied in our NSF work, which includes zinc, cadimum, cynide, phenol, cresol, benzene, chlorophenol, analine, and chloroform will be included on a microarray gene DNA chip. This would permit a broad base screening approach of gene expression for the detection of a broad range of chemical exposures. The second approach will evaluate specific gene expression using real-time quantitative PCR (RT-qPCR). This method would be more sensitive than the chip screening method, but will be more time intensive. The third approach will evaluate gene expression using reporter strains of Nitrosomonas europea that have been engineered using molecular methods to fluoresce when a specfic gene is expressed. We have developed reporter strains for heavy metal detection through the NSF grant that will be evaluated. This would provide for a low cost means of screening highway stormwater runoff that could be implemented in a standard water quality laboratory. A long term goal of the work would be the development of real time biosensors that could be directly employed in the field. This, however, would be beyond the scope of this work.

BENEFITS (more info)

The development of gene-based biosensors for monitoring stormwater runoff has the potential to reduce ODOT's stormwater monitoring costs and provides a powerful tool for use in stormwater management. A rapid, screening tool for quickly assessing the presence of various toxicants would allow ODOT to streamline it's monitoring efforts; detailed (and costly) characterization could be focused on stormwaters found during screening to contain toxicants. Biosensors could also be used in process control, signaling when contaminated stormwater needs to be diverted to treatment facilities prior to discharge. The proposed work would take advantage of knowledge gained in the past five years of funding through NSF. The work would focus on the practical application of gene based detection of chemicals and their potential toxicity in the environment. The work will consider mixtures of chemicals including metals and organics that can be present in a complex stormwater matrix. The methods are sensitive and compound specific. They are also based on the potential for the metal or organic to be transported inside a bacterial cell. Thus they are sensitive to whether a metal, for example, is in the charged form, where it is more easily transported into a cell and is more toxic, than in a particulate form. Thus, gene based expression of whole cells are likely a better sensor of toxicity that the monitoring of chemical concentrations and speciation. The development of a chip microarray approach would permit the analysis of many chemicals in one analysis. The costs of doing such an analysis is rapidly decreasing, so that a practical applcation is well within reach. The use of a reporter strain is an approach that can be utilized with low cost equipment, such as a fluorimeter. The research approach would easily permit the development of other gene based biosensors for chemicals that have not been evaluated in the current NSF project, for example copper. All the protocols have been developed to determine how to identify the sentile genes that are expressed in the presense of copper. Once the genes are identified they can easily be incorporated into the methods being tested. The testing of real stormwater samples would permit comparisons with other toxicity and chemical assays that are being developed. Thus, this project could complement other ODOT projects that are being pursued for monitoring stromwater runoff.

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