FLUOROMETRIC TISSUE-BASED BIOSENSORS

Oak Ridge National Laboratory

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PROJECT GOALS AND OVERVIEW

The two key goals of this project are development of water quality and inertially-hardened stand-off biosensors for detection of chemical and biological warfare agents under real-world combat situations. The sensors are based on the well-known principle that green algae and cyanobacteria emit a characteristic kinetic fluorescence signature that depends on their physiological status. Chemical and/or biological agents that interfere with the basic photosynthetic process will cause a change in the normal fluorescence signal. For airborne detection of CW agents such as tabun, the microalgae and cyanobacteria are entrapped on small fiber glass filter pads. Alternatively, for continuous monitoring of sun light-exposed primary drinking water, free-living indigenous algae or cyanobacteria may be used as the biosensor materials. Fluorescence spectroscopy can be performed with a small and relatively inexpensive optoelectronic measuring system. It is contemplated that inertially-hardened sensors that are capable of being launched or dropped into suspected danger areas can be constructed using these principles. We have demonstrated proof-of-principle for this system using tabun, tributylamine (a sarin stabilizer) cyanide and dibutylsulfide, a chemical analog of mustard gas. We have also expanded the original scope of the project by adding new tasks in the field of water quality testing. Working with primary drinking water samples from the Clinch and Tennessee rivers, we have detected herbicides and pesticides in direct-drawn samples of river water. Additional details are presented in the following sections.

ACCOMPLISHMENTS (SINCE LAST MEETING)

Airborne CW Agents and Simulants

Commercially available hand-held fluorometric detector systems were used to measure Photosystem II photochemical efficiency of green algae and cyanobacteria entrapped on filter paper disks. Toxic agents flowing in the gas stream through the sensors can alter the characteristic fluorescence induction curves with resultant changes in photochemical yields. Working at the Applied Chemistry Laboratory, Edgewood, MD and ORNL, signals were obtained for

- tabun (GA), 9, 10, 11 µg/L
- tributylamine (a sarin stabilizer), 7.4, 248, 642 µg/L
- dibutylsulfide (a mustard agent analog) 0.42, 2.9, and 5.2 µg/L

No signals were obtained for sarin (GB) or mustard gas (HD) at the test levels provided by the staff at the Applied Chemistry Laboratory: GB (6, 7, and 21 µg/L); HD (7, 8, 17, and 23 µg/L).

Water Quality Monitoring

Algae are common and normal inhabitants of surface waters and are encountered in every water supply that is exposed to sunlight. While a few of the algae are found in soil and surfaces exposed to air, the great majority are truly aquatic and grow submerged in the waters of rivers, ponds, lakes, reservoirs, streams, and oceans.

We have worked with primary fresh water drinking supplies from the Clinch and Tennessee rivers for development of tissue-based biosensors using naturally occurring aquatic photosynthesis as the sensing material. The Clinch is the main source of fresh drinking water for Oak Ridge, Tennessee while the Tennessee river is a major source for the city of Knoxville. We have successfully detected algae in every direct sample that we examined from the Clinch and Tennessee rivers and readily monitored changes in the characteristic fluorescence induction curves when the samples were exposed methylparathion, DCMU and cyanide. The unique aspect of this approach to real-time water quality monitoring is that, unlike conventional sensing devices, the sensor material is external to the detecting instrument and continuously refreshed. This solves the critical problem of sensor fouling and loss of activity.

PROJECTED MILESTONES (NEXT 12 MONTHS)

- Additional tests at the Edgewood, MD Applied Chemistry Laboratory with live CW agents
- Further development of water quality tissue-based biosensors
- Filing of patent applications
- Contact with potential industrial partners

ENVISIONED DELIVERABLES

(1) A relatively inexpensive stand-off biosensor capable of alerting U.S. military personnel to the presence of chemical warfare agents at levels that may be expected under real-world combat situations. (2) Use of indigenous aquatic photosynthetic organisms for continuous monitoring of primary drinking water.

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