Feature Article 3

Introduction

Buried explosive devices, such as landmines, generally have small plastic housings of extremely inexpensive construction that contain trinitrotoluene (TNT) or other explosive material mixtures. Landmines come in various sizes, shapes, and are designed to explode and maim whatever steps on the soil surface covering the mine. Afghanistan, Angola, and Cambodia are examples of countries that are littered with landmines deployed by invading military, government, and/or rebel groups. Most of the nations in this situation are those whose economies rely heavily on non-automated agricultural production, and the presence of landmines effectively removes large areas of arable land from agricultural production. The more obvious and urgent problem is that these mines kill and wound multiple people everyday. Those who live in the vicinity of minefields are normally aware of the existence of the minefield itself, but not of the specific locations of the mines that may be planted randomly, which could be as little as 10 meters apart. Since they are plastic, landmines cannot be located by metal detectors; in the developing world, the most common de-mining practice is that of a man with a stick. He will search for a landmine by feel, a practice that is imprecise at best and often a hideous short-term career at its worst. There is a clear and urgent need for the development of sensitive and specific explosive detection systems capable of mapping the presence of explosives in soil that can function in near real-time manner.

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With the support from the U.S. Army Engineer Research and Development Center – Topographic Engineering Center – Fluorescence Spectroscopy Laboratory via the U.S. Army Small Business Technology Transfer (STTR) search Agency, Agave BioSystems is developing a novel fluorescent system capable of detecting explosive residues. This system used antibodies and novel fluorescent quencher analogs to detect the presence of specific explosive residues.

Current Trace Explosive Detection Technology

Most of the current methods for analyzing soil for explosive contaminants involve chemical extraction of the explosives from a sample followed by complex analysis. The complexity of these techniques typically requires that the samples be moved offsite. These methods involve transferring soil samples to a laboratory and then using extraction techniques such as acetonitrile extraction [U.S. environmental Protection Agency (EPA) Method 8330] or supercritical extraction with CO2 (SC-CO2) (Halasz 2002). Once extracted, the samples are analyzed using either gas chromatography-mass spectrophotography (GC-MS), capillary electrophoresis- UV (CE-UV) or high pressure liquid chromatography –UV (HPLC-UV). Thus, these techniques require not only extensive handling of soil samples from contaminated
potentially explosive sites, but also expensive analytical equipment and highly skilled workers to perform all testing. Another test, the ETKplus kit, was developed by the Israel Institute for Biological Research and is currently used by Israeli police and other law enforcement agencies. This kit is currently sold in the U.S. by SecurityPro USA (Beverly Hill, CA). While this test is fairly sensitive and relatively simple to use, it is a qualitative, colorimetric assay that is based on text-book chemical reactions that were first described in the late 19th and 20th centuries. This system is prone to false positives based on the fact that alkali hydrolysis and Griess reactions used in the kit can cause non-explosive chemicals to form colored products similar to those generated by explosives like TNT, RDX, or nitrocellulose (Menzel 2004).

Previous Biological Solutions

Recently, over the past decade, genetically modified plants have been one of the focuses for biosensors. The idea is to have the genetically modified plants altered to uptake trace explosive materials and aid in the detection of land mines via a fluorescence or visual response when interrogated with an external light source. Genomic analysis of the response of Arabidopsis thaliana to trinitrotoluene and GFP have been studied (Stewart et al. 2003), but have yet proven success in the field. Also, there are logistical problems, which would require tending of potentially mine-laden fields or helicopter-based seeding over contaminated sites. In addition to plants used as biosensors, genetically modified microorganisms have been investigated for the potential to detect TNT. This was first demonstrated using Pseudomonas putida (Burlage 1999). This bacteria was engineered to contain a TNT inducible promoter fused to green fluorescent protein (GFP) and were tested on a faux minefield with surrogate landmines. Pseudomonas putida detected five of five landmines in a one-quarter acre plot; however, they also produced two false positive signals, indicating the presence of a landmine where none existed. There are several other drawbacks to a bacterial-based system. It requires that bacteria be grown and sprayed onto the minefield, which could be determined to be environmentally unacceptable. A government also might well object to the release of recombinant bacteria in the interest of national security. Additionally, it has been found that the bacterial signal is dependent on a plant substrate for bacterial colonization (Stewart et al. 2001). A number of bacterial bioreporters have been described that produce GFP in response to diverse chemicals such as arsenite (Stockt 2003), nitric oxide (Yin 2003) and TNT (Fischer 2000). While GFP may serve as a useful bioreporter in the laboratory setting, recent reports suggest that this reporter may not be suitable for detection of soil contaminants. For instance, Smith et al. (2002) demonstrated that expressed GFP produced high levels of fluorescence at pH 7.0, but at more acidic or alkaline pH, such as those likely to be encountered in potentially contaminated soil, fluorescence output was diminished.

Fluorescent Bioprobes System: BRITE MINE

BRITE MINE is a hybrid inorganic and organic approach for the detection of explosive agents within the environment. The advantages of this type of design are that the materials are not associated with recombinant DNA or genetic alterations and it is based on an environmentally stable silica (sand) construct. It is basically inert after use and is non-toxic to the environment. The detector that has been tested for TNT is both high sensitivity and high specificity, and the design can be altered so it can detect explosives such as RDX, HMX, and potentially others. Some disadvantages this detector may come across are that it is also biologically based and requires stabilization of antibodies in the environment. Thus, the inorganic silica-based microspheres containing nano-size holes are used for the encasement and protection of the antibodies. There also are potential signal to noise problems, which are being addressed and investigated within the Phase II of the study via use of fluorescence detector amplification or enhancement techniques. Finally, these materials will face certain challenges within the Phase II of testing for scaling up and production of these detectors over wide area applications. Field testing in a research mine field is planned to commence the summer of 2008. To visualize TNT detection in contaminated soil samples, a laser-induced fluorescence imaging (LIFI) system and a laser-induced fluorescent spectroscopy (LIFS) device were used to detect and measure fluorescence from the detecting particles. In these experiments, BioProbes were deposited on the surface of the contaminated soils, and the fluorescence emitted by the BioProbes was excited and detected at a standoff distance. The system used under the Phase I was tested at a distance of 1 meter. However, future design of the particle that would enhance or amplify the fluorescence signal and increase laser power output with the LIFI great distances could be reached. We are aiming for distances greater than 1000 meters. The developer of the LIFI’s system, Dr. Di Benedetto, states that “scaling remote sensing to airborne devices is critical for the successful detection of biological-based landmine detection systems and other real-time biosensors. Airborne laser-induced fluorescence
will provide remote access and direct evidence of specific contamination, whether it is TNT, heavy metals, or pathogens. It will bring a whole new dimension to remote sensing.” (Di Benedetto 1999).

Conclusions

The results presented above clearly demonstrate that Agave BioSystems, in collaboration with USRA and US Army ERDC, has developed a novel antibody-based quencher system for the stand-off detection of low concentrations of the explosive TNT. Incorporating anti-TNT antibodies and a fluorescence quencher molecule bound to a TNT analog into novel porous silica microspheres will result in a rugged assay suitable for field use. In developing the novel silica microspheres, fluorescent anti-TNT antibodies, the non-fluorescing quencher molecule, and using these components to detect a wide range of TNT concentrations, proof-of-concept was successfully demonstrated for this explosives detection system, which involved the innovative method using fluorescent antibodies encapsulated in porous silica microspheres for stand-off detection of explosives. In addition, the concentration limits of fluorescent detection of TNT were down to 10–6 M or 0.2 ppm. Agave BioSystems and USRA are now poised to extend the development of a sensitive and robust explosive detection system for the stand-off detection of a variety of military explosives in the Phase II. Dr. Clint Smith of the U.S. Army ERDC- TEC-Fluorescence-Spectroscopy- Laboratory is setting up the field test sites for the future Phase II testing in 2008 with NEWTEC Inc. Future efforts will focus on scale up of materials, developing assays to detect multiple explosive agents using multiple spectral wavelengths, and adapting the assay for stand-off detection of explosive materials under field conditions. In addition, this research effort will lead to other explosives detection applications such as identification of post-conflict landmine proliferation, improvised explosive devices (IEDs), and IED production facilities.

Literature Cited


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