Utilization of Naturally Occurring Biosensors to Detect Toxins in Our Nation's Water Supply

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Abstract

The events of September 11, 2001 have forever changed the way this Nation views security and the threat to its citizens. One area where this increase in security is clearly needed is that of protecting our Nation's water supplies against malicious or even unintentional contamination by chemical toxins. Detection of these chemical toxins historically has been determined by point tests of water samples according to a prescribed test cycle.

The use of biosensors to detect chemical toxins on a continual basis has been a desired goal for years, and the bases of methods to accomplish this have taken many forms ranging from multi-cell organisms such as fish to single cell cultured algae. All of these methods have had similar drawbacks, primarily that the biosensor needed to be cultured or the test agents were not naturally occurring within the water sample being processed.

An innovative biosensor is presented that overcomes the limitations of current technologies by utilizing naturally occurring wild algae as the test agent. Through the use of fluorescence and a sophisticated software algorithm capable of adaptively monitoring multiple variables in realtime, it is now possible to continuously detect the presence of toxins through the use of a stand-alone automated system. This paper will discuss the science of algae florescence and the impact of toxins on it, address suitability of the testing process for a variety of source water types, and present an overview of an approach for utilizing this technology to provide an automated continuous alert system for detecting toxins which would be a significant step toward securing our Nation's source water supply.

Over the past century, there has been an increased awareness that the raw surface water, that many of us take for granted, may contain harmful chemical toxins, eventually ending up in our households when we turn on the tap. Many of the surface water supplies of our Nation have been, and still are used for recreational activities. This easy and public accessibility poses many potential hazards. Since the eleventh of September, there has been a dramatic increase in the concern over the possibility of toxins introduced in the source water supply, whether done accidentally or done with malice and forethought.

The water monitoring industry, as well as the Federal Government, has long searched for a simple biosensor that could be used to detect toxins in water supplies, both quickly and easily. Several types have been used, and continue to be used in the industry, ranging from the simple cultured algae to the more complex multi-cell organisms such as daphnia and blue gill fish. All of these biosensors have similar drawbacks, either they need to be lab cultured, need to be maintained, or need to be continually replenished. The desire to use a simple, quick reacting, naturally occurring biosensor has been a goal for many years. There are four main challenges that must be resolved to utilize a simple naturally occurring biosensor: identification of a commonly occurring viable biosensor, establishment of a reliable method for monitoring and collecting data on the biosensor, protocols for the analysis and interpretation of the data collected, and packaging the system so that it could move out of the lab environment and into the field.

Any one who has ever raised tropical fish is probably aware how sensitive aquatic life is to the introduction of toxins in the water. Thus it is not surprising that science has looked to a multitude of naturally occurring aquatic biosensors. One drawback to most of them is that what is naturally occurring in one geographic location may not apply to other locations, especially when a biosensor is sought that exists around the world.

In trying to identify a simple naturally occurring biosensor, algae and cyanobacteria, which are present in all surface, waters exposed to sunlight quickly emerge as the most likely candidate. The unattached microorganisms that are found individually or in small clumps floating in rivers and lakes are composed of phytoplankton and zooplankton. Most of the phytoplankton is composed of algae (Palmer, 1959). Based on cell count, biomass, and photosynthetic carbon fixation, green microalgae is a major component of the phytoplankton population. Algae are ubiquitous, found in every surface water body in the world, and can grow year-round in all climatic conditions, and survive on nutrients in low concentrations or in forms that are unsuitable for larger multicell organisms (Happey-Wood, 1988). The well understood properties of algae, its availability the world over, its simple cell structure, and its ability to survive in areas where the lack of nutrients may pose an obstacle to larger celled organisms make it an excellent choice for meeting the first challenge. With the identification of a naturally occurring biosensor the second challenge can be addressed.

By the early 1960's scientists have understood and documented the various processes that occur as part of the normal photosynthetic activity of green plants exposed to solar radiation. The incident light energy is harvested by a network of *antennas*, which are made up of large number of pigment molecules bound to proteins that provide for the appropriate orientation and inter-positioning of the pigment molecules. The absorbed energy is transferred from peripheral antenna complexes to complexes near photosynthetic reaction centers where the energy conversion can occur. Typically the light harvesting antennas are optimized to maintain a high electron transfer rates even at lower light intensities. At higher intensities the amount of light that is absorbed by plants easily exceeds the capacity of electron transfer initiated by the reaction centers. A very small portion of the energy absorbed in excess of the photosynthetic capacity of the plant is converted into heat, while the majority of this excess energy is re-emitted or fluoresced from the plant. Over the past few years, scientists and engineers have made further progress in refining and understanding the principles of fluorescence and in measuring the output. By combining dark-adapted measurements with those collected in the presence of light a very detailed analysis of the photosynthetic process can now be preformed (Maxwell and Johnson, 2000). Figure 1 shows a standard fluorescence curve for algae.

The state of technology has increased during the past several years to allow for the wide scale availability of highly sensitive fluorometers capable of reliably measuring fluorescence of algae with ever increasing resolution. These devices have provided a quick and reliable method for collecting the fluorescence data in lab environments. With the increased understanding of the fluorescence and the ability to reliably collect the data, the second challenge has been addressed.

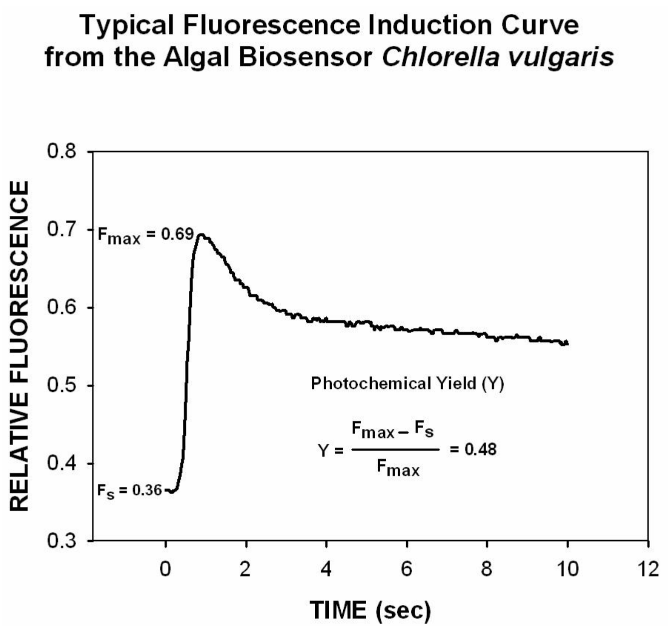

Figure 1

Through research, scientists at Oak Ridge National Laboratory (ORNL) have discovered that the introduction of toxic agents will significantly alter the expected fluorescence curve that would be anticipated from naturally occurring fresh water algae. ORNL studies have been conducted on methyl parathion (MPt), potassium cyanide (KCN), diuron (DCMU), and paraquat. Figure 2 shows both a standard fluorescence curve for algae and a curve for potassium cyanide. These changes in the fluorescence are due to the fact that the toxic agents block electron transport, impair light energy transfer, or generate toxic photoproducts (Rodriguez Jr. et al, 2002).

Utilizing the ORNL patented technology, entitled "Tissue-Based Water Quality Biosensors for Detecting Chemical Warfare Agents," (inventors Elias Greenbaum and Charlene A. Sanders. Patent Application 2002/0102629.) Researchers at United Defense LP, have developed extensive software that is capable of analyzing the fluorescence curves of algae in real-time to assess the probability of a toxin being present. This understanding of the influence of toxins on a fluorescence curve and the capability of being able to correctly interpret the data and assess the probability of a toxin being present provide a solution for the third challenge.

The last challenge was how to move a system that utilizes a simple naturally occurring bio sensor out of the lab environment and utilize it in the field. The need for field deployable around the clock toxin monitoring capability is obvious when someone considers three key factors.

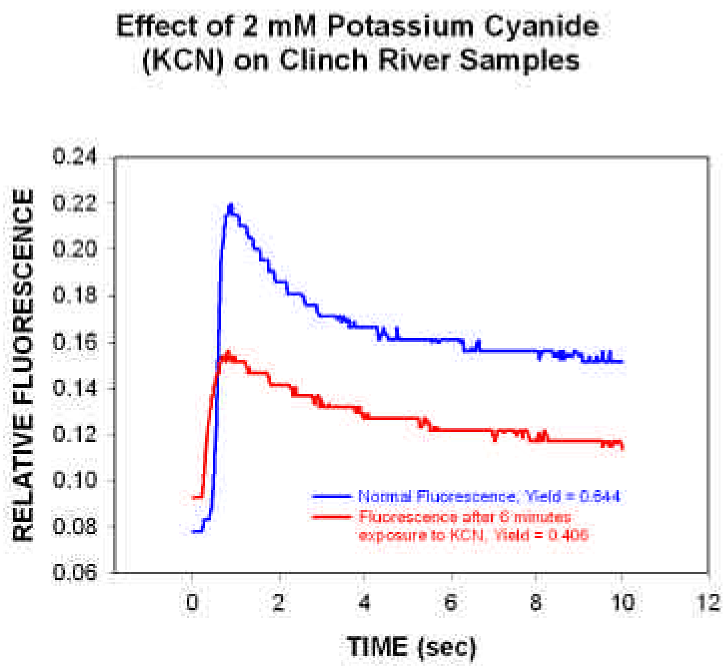

Figure 2

The first, as stated earlier, is that most of the raw surface water supplies in America are public accessible. Second is that the agency responsible for testing/monitoring the source water is very often remotely located. Lastly, the size of most of the source surface water entities make it almost impossible for human monitoring of the entire area, thus allowing toxins to be introduced into the water without someone observing the initial insertion of the contaminate. Consequently the ideal system would be one that can be located at the water source, have the ability to communicate over large distances from remote locations, and be continuous and autonomous in operation. Figure 3 illustrates this concept where several systems monitor the reservoir and communicate wirelessly with the central monitoring station in the city.

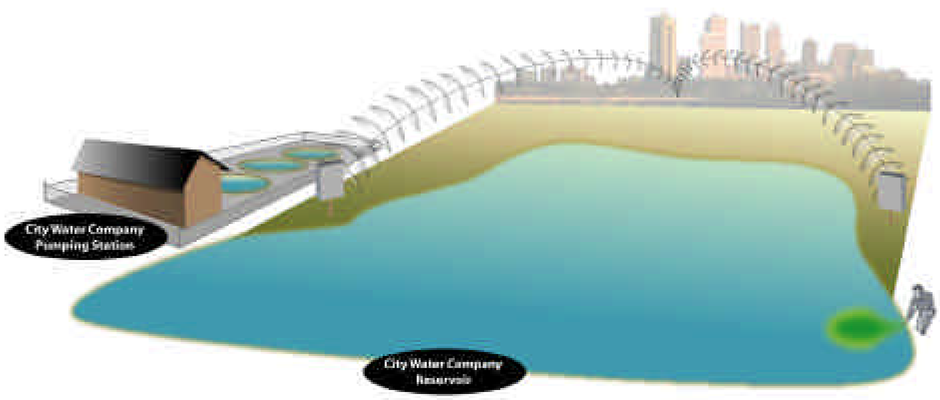

Figure 3

Scientists at United Defense LP have developed a product called WaterSentry™ that meets all the challenges of being a field deployable system. WaterSentry™ offers a continuous and rapid reagentless broad-trigger detection capability against water-borne toxins by evaluating the natural activity of green algae. The technology is capable of detecting the presence of toxic agents at levels expected to be encountered during military conflicts or terrorist attacks on civilian water supplies; it is not intended for precise chemical analysis to determine the exact agent identification. This device utilizes patented AquaSentinel℠ technology from ORNL. Figure 4 shows the basic concept of how WaterSentry™ works.

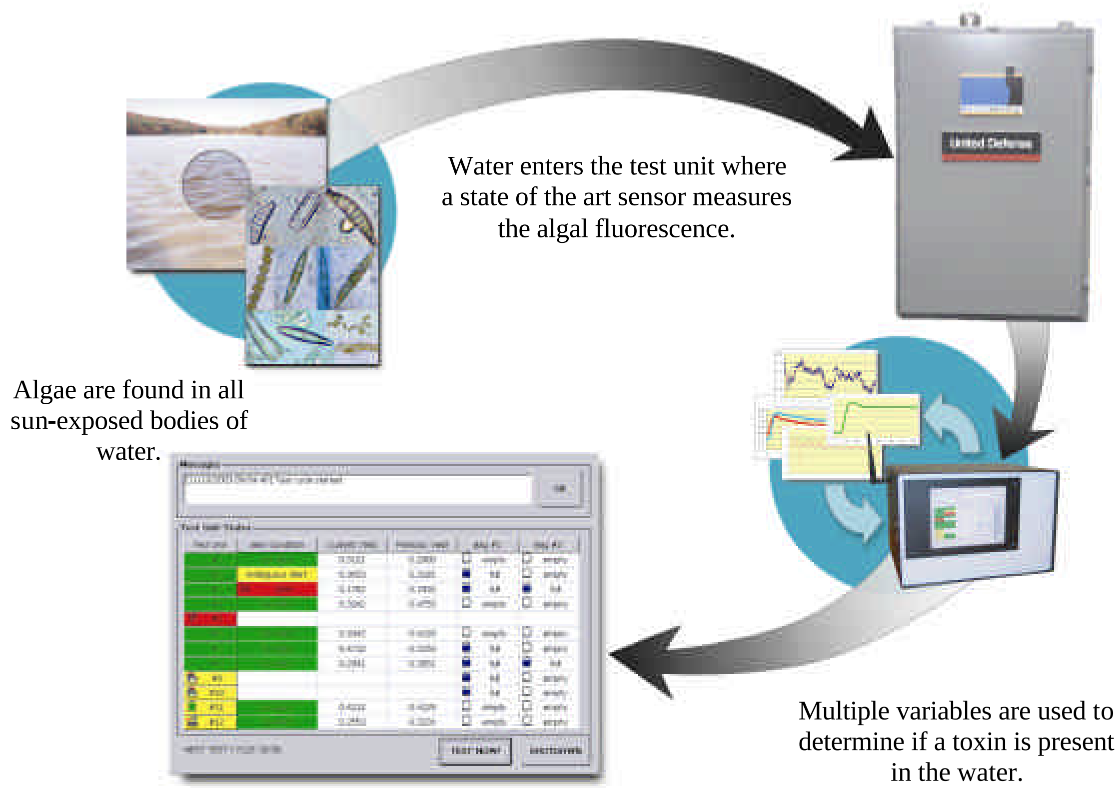

Figure 4

The WaterSentry™ system is capable of integrating multiple test units into a single control station, such that large distributed source water supplies can be monitored from a central location. This technology could be used with purified water in which the natural algae have typically been killed by reintroducing small amounts of cultured algae.

WaterSentry™ works by monitoring the optoelectronic fluorescence of the algae. As stated earlier in the paper, fluorescence detection and monitoring is a well-understood process. By measuring the natural algae fluorescence and establishment of a baseline of activity, WaterSentry™ can detect any deviations from the baseline. These deviations indicate that the algae are being stressed in some manner, perhaps including the presence of toxins in the water. The operational concept for WaterSentry™ is that detected deviations will alert the water supply control staff that water use from this source should be immediately stopped and a detailed chemical analysis performed.

Experiments using methyl parathion (MPt), potassium cyanide (KCN), paraquat and Diruron® (DCMU) with natural water from the Clinch River in Tennessee showed that WaterSentry™ can reliably detect toxin presence within minutes. Each fluorescence activity check takes ten seconds or less, so the time to detect is dependent upon the frequency and time interval between checks. Additional experiments with MPt, KCN, and DCMU using water from Tiberia Lake in Israel yielded comparable toxin detection capability.

While the WaterSentry™ technology is effective in detecting chemical toxins in water, biological agent detection may be more difficult. A limited set of experiments in detecting biological agents has been conducted by ORNL with some rather promising results. The test data shows that while algae may not quickly react to the biological agent itself, chemicals often associated with biological agents do trigger a detectable reaction. Experiments have been conducted with the Ricin, found in the possession of suspected terrorists in the U.K., and have shown that the reducing agent Dithioerythritol affects algae to a detectable level by the WaterSentry™. In addition, we have found that Aflatoxin B1, a toxin produced by certain types of fungi, results in a detectable change. The effectiveness of our technological approach to detecting water-borne toxins has been shown in recent research (Rodriquez, *et al,* 2002.)

In summary, WaterSentry™ is designed and proven to work with and markedly improve the effectiveness of high-end water analysis laboratory measurements with several important features and benefits:

- Unlike currently available bench-top biosensor technology, the United Defense WaterSentry™ technology provides a field deployable instrument.
- WaterSentry™ is capable of detecting variations in algae activity within minutes, providing an immediate alert.
- Because there is an unlimited supply of biosensor material (algae) in the monitored environment, continuous around-the-clock monitoring of primary-source drinking water is possible with this approach.
- An early warning alert of toxic agents is provided by the short turnaround time needed for analysis.

References

Happey-Wood, C.M., 1988. Ecology of freshwater plantonic green algae. In: Sandgren, C.D. (Ed.), Growth and Reproductive Strategies of Freshwater Phytoplankton. Cambridge University Press, New York, pp. 175-226.

Maxwell K., Johnson, G.N., 2000. Chlorophyll fluorescence-a practical guide. J. Exp. Bot. 51, 659-668.

Palmer, C. M., 1959. Algae in water supplies, Public Health Service Publication No. 657. US Department of Health, Education, and Welfare, Cincinnati.

Rodriguez , M., Jr., Sanders, C.A., Greenbaum, E., 2002. Biosensors for rapid monitoring of primary-source drinking water using naturally occurring photosynthesis. Biosensors & Bioelectronics 17, pp. 843-849.