Journal of Aquatic Ecosystem Health 3: 87–92, 1994.
M. Munawar (ed.), Aquatic Ecosystem Health and the Ecological Significance of Bioassay Techniques.

Automated biomonitors – first line of defense

D. Gruber, C. H. Frago & W. J. Rasnake
Biological Monitoring, Inc., P.O. Box 184, Blacksburg, VA 24063, USA

Key words: automated biomonitors, biological monitors, early warning systems, toxics monitoring, water quality monitors

Abstract

Automated biomonitors operate on a real-time basis and utilize living organisms as the sensors. Traditionally, chemical monitors have been used to assess water quality. However, biological monitors respond to a greater number of toxic conditions. An overview of the various automated biomonitors, assessed by the types of biological sensors employed, is presented. The sensors used include bacteria, algae, invertebrates, and fish. Of all the systems, those monitoring the ventilatory behavior of fish have evolved the furthest with respect to their research, development, commercial availability, and field testing.

1. Introduction

Automated biomonitors operate on a real-time basis and use living organisms as the sensors, ideally providing a continuous flow of information regarding water quality. Theoretically, they are early warning systems and upon the detection of a toxic event, alarm conditions should be capable of being transmitted both locally and to remote locations (e.g., an operation's command post). Such systems must be sensitive and reliable, providing the absolute minimum of false alarms. When integrated with continuous and on-line chemical monitors, they provide a creditable first line of defense to protect wastewater receiving systems and/or drinking water treatment plant intakes.

Traditionally, chemical monitors have been used to assess water quality. Over the years, the emphasis on these monitors has led to highly reliable and sensitive systems, incorporating sophisticated and innovative technologies. In contrast, automated biomonitors have lagged behind in these categories as well as in their wide-spread applications, simplicity of use, and clear-cut quantification and interpretation of the data generated.

Increasingly, the limitations of chemical monitoring for assessing water quality are becoming recognized. Although these techniques are invaluable for identifying the causative agents of a toxic condition, they rarely are, or can be used to detect the occasional presence of one or more of a vast array of potentially toxic conditions. For the most part, chemical monitors are very specific, capable of detecting the presence of only one given chemical per technique. Furthermore, there are only a handful of such monitors which are capable of operating automatically, on-line, and on a real-time basis. More often than not, the techniques involved require highly trained and skilled technicians. In addition, the costs involved become prohibitive when considered for applications in continuous, real-time monitoring programs. For example, the average cost for analyzing for the 126 U.S. Environmental Protection Agency (EPA) priority pollutants approaches $1000 (U.S.) per sample. Further complicating such applications is the fact that many chemical monitors are not capable of detecting the presence of chemicals at the low levels where toxicity occurs, at microgram or less concentrations. Finally, and by far the most significant limitation of chemical monitors, is that simply knowing the identities and concentrations of the chemicals present in a water sample is rarely enough to predict toxicity, which is from the point of view of the organism in its particular environment.

In the U.S., these limitations have been recently accepted by the regulatory communities. For example, the U.S. EPA to demonstrate compliance with the Clean Water Act now requires an integrated monitoring approach, consisting of both biological and chemical assessments of wastewaters (e.g., U.S. EPA, 1991). Furthermore, most recently, the U.S. EPA has emphasized that the same concentrations of many chemicals, especially metals, likely differ in net toxicity from one wastewater receiving system to another. This emphasis has been demonstrated by the publication of guidance documentation to modify water quality criteria, and thus wastewater permit limits, on a site-specific basis by employing biological monitoring techniques (U.S. EPA, 1992).

Although biological monitors have traditionally lacked technological innovations, their applications for assessing water quality is certainly conceptually appealing as compared to chemical monitors. This is true because living organisms are general sensors of toxicity. Regardless of the nature, number of concentration of the chemical constituents present, if conditions are toxic, living organisms respond accordingly. Living organisms assimilate all the environmental cues and stimuli present; their responses may range from being considered extreme (e.g., death) to subtle (e.g., changes in their ventilatory behavior).

2. Current status

Automated biomonitors for assessing water quality are rapidly evolving. Nonetheless, both the availability and actual on-site applications of automated biomonitors are few since unfortunately, most automated biomonitors remain in the prototype phase of development. The evolution into a production phase is often hampered by a lack of capital investment. More often than not, this lack of capital investment reflects a reluctance, and even an inability, of the research biologist to understand, communicate with, and motivate the financial community. This is in part compounded by a lack of understanding of biological systems by decision makers, who in this field, have traditionally been engineers and chemists who because of their training had a tendency to use chemical and physical methods only, neglecting biological methods (Cairns, 1990).

Several automated biomonitors have successfully, albeit modestly evolved into limited versions of production models with actual on-site applications. There are several explanations for their limited success. The most significant ones reflect both the insight of governments and/or the financial communities, and their subsequent investments in the technologies. For example, one system has successfully evolved largely because of the Small Business Innovation Research (SBIR) grant program of the United States government (Gruber *et al.*, 1991). This SBIR grant program typically provides up to $300,000 (U.S.) to successful small business applicants in two phases, enabling 2.5 years of research and development efforts. Significantly, in order to receive more than 80 percent of the funds, the small business must have a formal agreement for matching funds, typically from the financial and/or manufacturing communities, dependent upon the successful completion of the research and development objectives.

3. Desirable traits

In order to gain acceptance, automated biomonitors must possess a number of desirable characteristics. Table 1 presents 22 of the most desirable traits of automated biomonitors. These traits range from ease of operation to cost effectiveness. It is no coincidence that the automated biomonitors possessing the greatest number of these characteristics represent those systems operating on-line at the most number of sites. As the demand for automated biomonitors increases, the technologies will undoubtedly continue to improve. This combination will yield a vast array of sensitive automated biomonitoring systems that will reliably protect our water resources. In order for this to occur, the systems must consider the end user who is typically on operator/technician with very little formal education. In addition, environmental and/or civil engineers will be directly responsible for the operator/technician. The need for biologists must be limited to the initial training aspects during the installation phase of the on-site application.

4. Types of sensors

The major characteristic distinguishing automated biomonitors from each other is the organism selected for use as the sensors. The sensors include bacteria, algae, invertebrates, and fish. Almost exclusively the systems employ a single-species approach for assessing water quality. Table 2 presents a variety of systems presented by the type of sensors employed. A detailed review of many of these systems has been presented by Gruber (1988).

Table 1. Desired characteristics of automated biomonitors

Truly automated & real time	Minimal training needs
Operates on-line	Compatible with industrial environments
Sensitive	Easy to operate
Rapid and nearly instantaneous response	Minimal operating expenses
Easily interpretable alarms	Minimal maintenance expenses/requirements
Reliable alarms	Sensor replacements readily available
Minimal false alarms	Inexpensive sensors
Reliable technology	Real-time remote transmission of *all* data
Commercially available with support	Immediate remote and local alarm notification
Inexpensive	Integrates biological alarms physically and logically with chemical monitors
Minimal installation requirements	Automatically collects water samples

Table 2. The various automated biomonitors, listed by sensor type, comparing their concepts of operation and ease of use.

Sensor type	Response variable	Method of detection	Level of develop. (1 = Min 3 = Max)	Degree of automation (1 = Min 3 = Max)	Operator skill required (1 = Min 3 = Max)
Algae	Fluorescence	Photomultiplier	2	2	3
	Photosynthesis	Amperiometric Electrode	2	2	3
Bacteria	Activity	CO_2 Electrode	1	1	3
	Bioluminescence	Photomultiplier	3	2	2
	Nitrification	D.O./NH_3 Electrode	3	2	2
	Respiration	D.O. Electrode	2	2	2
Zooplankton	Activity	Infrared Video	2	1	3
Insects	Activity	Non-invasive electrodes	2	1	2
	Respiration	D.O. Electrode	2	2	2
Daphnids	Locomotion	Video/Photocells	2	1	2
	Respiration	D.O. Probe	1	1	3
Bivalve molluscs	Valve position	Electromagnetic coils	3	3	1–2
Fish	Electrical discharge	Electrodes	1	1	3
	Locomotion	Video-Photocells	2	1	3
	Preference-avoidance	Video	2	2	3
	Rheotaxis	Photocells	3	3	1
	Ventilation	Non-invasive electrodes	3	3	1

4.1. *Bacteria*

Investigators of automated biomonitors have examined a variety of bacterial response variables for detecting toxicity. However, only a few approaches have yielded technologies capable of on-site application. Of these, there are four distinctly different bacteria-based systems that stand out, namely, systems which monitor oxygen consumption, nitrification, CO_2 production, and bioluminescence. A major difference between these units is the actual location of the bacterial sensors; they may be free living in exposure cells, fixed to a substrate (biofilter), or immobilized within electrodes. The systems which monitor oxygen consumption, often referred to as bacterial respirometers, have been operating at sites continuously and on a real-time basis longer than any other type of bacterial sensors (e.g., Martin, 1988). These respirometers incorporate dissolved oxygen probes to measure changes in oxygen consumption by bacteria exposed to the water being monitored. The systems do not provide for an automated alarm condition, instead requiring site specific decision-making judgement by the operators.

Holland & Green (1975) demonstrated one of the earliest attempts to use bacteria as a monitoring tool. Their approach was to monitor a mixture of *Nitrosomas* and *Nitrobacter* on a granite chip substrate/biofilter for the inhibition of nitrification. Some use technologies to assess general activity by measuring CO_2 production (Dorward & Barisas, 1984). Unfortunately, these two approaches have lacked the technological developments necessary for truly on-line and automated biomonitoring.

The microbial systems which monitor for bacterial bioluminescence have been operating exclusively on a discrete basis, versus automated and continuous operation for a number of years. The systems attempt to correlate changes in the bioluminescent behavior of a marine bacterium through exposure routines employing serial dilutions (Bulich, 1979). Although, numerous applications of these discrete monitors abound, their acceptance by the regulatory community in the U.S. has been almost non-existent. These systems are cited here, because reportedly two have been recently developed for near-continuous mode operations (e.g., Lumistox/Microtox). Through the use of robotics technology, samples are collected, and the needed dilutions are prepared automatically every 30 minutes. However, it is reported that a single system will require a minimum of $30,000 (U.S.) annually for materials alone.

4.2. *Algae*

Optoelectric technology is typically incorporated into algal based systems which, for example, monitor fluorescence to estimate density and distribution (Benecke *et al.*, 1982). In another system, the illumination of a biocatalyst and oxygen production of eukaryotic algae are used to monitor photosynthetic events. These approaches are reportedly being researched and developed commercially in France and Germany. Field testing and commercial availability of these systems are relatively unknown.

4.3. *Invertebrates*

As seen in the accompanying table (Table 2), several different types of aquatic invertebrates have been used as sensors, with varying success, to develop automated biomonitors. The response variables monitored for all include some form of either locomotor or respiratory activity. For example, in the daphnid monitor, developed in the United States, video cameras correlate toxicity with locomotor activity. Smith & Bailey (1988) have incorporated infrared light source and photocells to detect the presence of moving daphnids.

In the Netherlands, the valve movements of mussels are used to assess toxicity (Kramer *et al.*, 1989). In this system, the underlying assumption is that mussels will close their shells under environmental stress. The position of both valves are automatically monitored by means of an electromagnetic field induced by coils placed on the valves of eight, individually monitored mussels. This system is commercially available and is currently undergoing trials on the Rhine River in the Netherlands. Other invertebrate based systems have included aquatic insects and have employed fiber optic technologies (Batac-Catalant White, 1983) and electrical impedance techniques (Morgan *et al.*, 1988; Heinis *et al.*, 1990).

4.4. *Fish*

By far, the greatest number of automated biological monitors undergoing research and development have used fish as sensors. The response variables monitored have included locomotor activity, rheotactic behavior, electric organ discharge, and ventilatory behavior.

The fish locomotor monitors employ video techniques to assess either individual or groups of fish exposed to the water source in question. The systems have been under investigation in the United States for a number of years (e.g., Smith & Bailey, 1988). However, the concept of an automated alarm has yet to be developed and integrated into these systems.

The rheotactic behavior types of automated biomonitors typically assess the ability of a fish to maintain its position in a moving stream of water (e.g., Poels, 1975; Morgan, 1977). Photocells are often applied to detect when a fish has drifted downstream in a trough. Subsequently, the animal is shocked, thereby training it to remain upstream. The ability of a fish to remain upstream is believed to be affected by the presence of toxicants, causing swimming impairment. Such systems have been developed in Germany and Ireland with field testing having been conducted in these countries as well as in the United Kingdom.

A related approach assesses fish preference-avoidance behavior by placing fish in a so-called fluvarium test system. Within these test chambers, fish are offered a choice of locations, either a control water or the water supply in question. The assumption is that fish should avoid the locations fed with the water source of poor quality (Sprague, 1964; Van Hoof, 1980). Detection of the position of the fish has been accomplished by optical technologies utilizing photocells (Kleerekoper *et al.*, 1975) and/or video camera (Korver & Sprague, 1988).

Germany and the United Kingdom are also responsible for the research and development of an automated biomonitor assessing the electric organ discharges of weakly electric fish (Mormyrids & Gymnotids). These systems track the pulse frequencies generated by groups of fish. They represent the newest of developing automated biomonitoring systems. Reportedly, the techniques hold promise, but applications to date are extremely limited (Geller, 1984; Ewen, 1987).

Of all the automated biomonitors, those assessing fish ventilatory behavior utilize the most innovative electronic and computer technology and have the greatest number of research, development, and commercial applications. Field testing has been conducted in Australia, Canada, Germany, the United Kingdom, and the United States. In these systems, non-contact submerged electrodes are typically utilized to receive the microvolt bioelectric signals as generated by individual fish. State-of-the-art electronics are used to filter and amplify the signals which are then interfaced with computers. In the system commercially developed in the United States (Bio-Sensor®), two models are available; one using 8, and the other 12 fish, individually monitored fish. This system has been compartmentalized into a relatively compact cabinet to facilitate on-site applications. Interfacing the biological monitor with a variety of chemical monitors has been accomplished. Alarm generation is automated and notification occurs at both local and-or remote sites. Field testing has been relatively extensive compared to others, but overall it is still considered to be limited (Gruber *et al.*, 1991).

5. The future

Automated biomonitors, overall, remain in their infancy with their development and acceptance. Their development will increase as they become more accepted. The limited acceptance primarily reflects the lack of communication between the biologists developing such systems and others representing the community of water quality specialists, in particular, engineers and policy makers. In a field traditionally dominated by engineers and chemists, biologists must learn to see beyond their laboratory environment and be able to explain their rationale in terms others can understand. They must become more pragmatic and actively involved in the development of standard methods of testing and the implementation of Best Available Technologies (BAT).

Whereas no single species monitoring device may be capable of predicting toxicity to a community of organisms in wastewater receiving systems and, at the same time, to humans about to drink from water supplies, they do represent the best alternative currently available. These systems will respond to the greatest number of toxic compounds. Some of the systems (e.g., fish ventilatory behavior monitors) even lend themselves to a multispecies approach, thereby minimizing the single species argument. However, this approach has yet to be applied.

There is little doubt that automated biomonitors will continue to evolve. Biologists must stress the simplification of these technologies. When coupled with automated water sampling devices for either on-line or subsequent chemical monitoring, automated biomonitors represent the most creditable first line of defense for assessing water quality.

Acknowledgements

The authors wish to express their extreme gratitude to the National Science Foundation's Small Business Innovative Research (SBIR) grant program (Grant No.

III – 9223109). It is recognized that without this support, this present endeavor could not have been undertaken.

References

Batac-Catalan, Z. & D. S. White, 1983. Effects of chromium on larval chironomidae as determined by the optical-fiber light interruption biomonitoring systems. In: W. E. Bishop, R. D. Cardwell & B. B. Heidolf (eds), *Aquat. Toxicol. & Haz. Assess.: Sixth Symposium*. pp. 469-481. ASTM, Philadelphia.

Benecke, G., W. Falke & C. Schmidt, 1982. Use of algal fluorescence for an automated biological monitoring system. Bull. Environ. Contam. Toxicol. 28: 385–395.

Bulich, A. A., 1979. Use of luminescent bacteria for determining toxicity in aquatic environments. In: L. L. Marking & R. A. Kimerle (eds), Aquat. Toxicol. ASTM STP 667: 98–106.

Cairns, J. Jr., 1990. The genesis of biomonitoring in aquatic ecosystems. The Environmental Professional 12: 169–176.

Dorward, E. J. & B. G. Barisas, 1984. Acute toxicity screening of water pollutants using a bacterial electrode. Environ. Sci. Technol. 18: 967–972.

Ewen, R., 1987. *Biological Testing for Toxicity Control in Open Waters*. Endress & Hauser, Germany.

Geller, W., 1984. A toxicity warning monitor using weakly electric fish, *Gnathonemus petrsii*. Water Res. 18: 1285–1290.

Gruber, D., 1988. A historical perspective. In: D. Gruber & J. M. Diamond (eds), *Automated Biomonitoring: Living Sensors as Environmental Monitors*. pp. 15–20. Ellis Horwood Ltd., Chichester.

Gruber, D., J. M. Diamond & M. J. Parson, 1991. Automated biomonitoring. Environ. Auditor. 2(4): 229–238.

Heinis, F., K. R. Timmermans & W. R. Swain, 1990. Short-term lethal effects of cadmium on the filter feeding chironomid larva *Glyptotendipes pallens* (Meigen) (Diptera). Aquat. Toxicol. 16: 73–86.

Holland, G. J. & A. Green, 1975. Development of a gross pollution detector: Laboratory studies. Water Treatment Examination 4: 81–99.

Kleerekoper, H., D. Gruber & J. Malis, 1975. Accuracy of localization of a chemical stimulus in flowing and stagnant water by the nurse shark, *Ginglymostoma cirratum*. J. Comp. Physiol. 98: 257–275.

Korver, R. M. & J. B. Sprague, 1988. A real-time computerized video tracking system to monitor locomotor behavior. In: D. S. Gruber & J. M. Diamond (eds), *Automated Biomonitoring: Living Sensors as Environmental Monitors*. pp. 157–171. Ellis Horwood Ltd., Chichester.

Kramer, K. J. M., H. A. Jenner & D. Zwart, 1989. The valve movement response of mussels: A tool in biological monitoring. Hydrobiologia 188/189 (Dev. Hydrobiol. 54): 433–443.

Martin, J. V., 1988. Biomonitoring of polluted waters: Three systems. In: D. S. Gruber & J. M. Diamond (eds), *Automated Biomonitoring: Living Sensors as Environmental Monitors*. pp. 172–181. Ellis Horwood Ltd., Chichester.

Morgan, E. L., R. C. Young & J. R. Wright, 1988. Developing portable computer-automated biomonitoring for a regional water quality surveillance network. In: D. S. Gruber & J. M. Diamond (eds), *Automated Biomonitoring: Living Sensors as Environmental Monitors*. pp. 127–144. Ellis Horwood Ltd., Chichester.

Morgan, W. S. G., 1977. An electronic system to monitor the effects of changes in water quality on fish operulum rhythms. In: J. Cairns, K. L. Dickson & G. F. Westlake (eds), Biological Monitoring of Water and Effluent Quality, Amer. Soc. Test. Materials, Spec. Tech. Pub. 607: 38–55.

Poels, C. L. M., 1975. Continuous automatic monitoring of surface water with fish. Water Treatment Examination 24: 46–56.

Smith, E. H. & H. C. Bailey, 1988. Development of a system for continuous biomonitoring of a domestic water source for early warning of contaminants. In: D. S. Gruber & J. M. Diamond (eds), *Automated Biomonitoring: Living Sensors as Environmental Monitors*. pp. 182–205. Ellis Horwood Ltd., Chichester.

Sprague, J. B., 1964. Avoidance of copper-zinc solutions by young salmon in the laboratory. J. Water Pollut. Control Fed. 36: 990–1004.

U.S. EPA (United States Environmental Protection Agency), 1991. Technical Support Document for Water Quality-based Toxics Control. Off. Water Regulations and Standards, Washington, D.C. EPA/505/2-90-001.

U.S. EPA, 1992. Guidance on Interpretation and Implementation of Aquatic Life Criteria for Metals. Off. Sci. Technol. Washington, D.C.

Van Hoof, F., 1980. Evaluation of an automatic system for detection of toxic substances in surface water using trout. Bull. Environ. Contam. Toxicol. 25: 221–225.