



Review

Microbial biosensors for recreational and source waters[☆]

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ABSTRACT

Biosensors are finding new places in science, and the growth of this technology will lead to dramatic improvements in the ability to detect microorganisms in recreational and source waters for the protection of public health. Much of the improvement in biosensors has followed developments in molecular biology processes and coupling these with advances in engineering. Progress in the fields of nano-engineering and materials science have opened many new avenues for biosensors. The adaptation of these diverse technological fields into sensors has been driven by the need to develop more rapid sensors that are highly accurate, sensitive and specific, and have other desirable properties, such as robust deployment capability, unattended operations, and remote data transfer. The primary challenges to the adoption of biosensors in recreational and source water applications are cost of ownership, particularly operations and maintenance costs, problems caused by false positive rates, and to a lesser extent false negative rates, legacy technologies, policies and practices which will change as biosensors improve to the point of replacing more traditional methods for detecting organisms in environmental samples.

Microbial biosensors and the conceptual components of these may be defined in multiple ways. Microorganisms, or organism too small to reliably be identified by the naked eye, include viruses, bacteria, protozoa, cyanobacteria, fungi, and microflora and microfauna such as some species or life cycle stages of algae and arthropods. Sensors are signal transduction devices which send a signal to a reporter in response to a change in state in the environmental milieu where they are deployed. A sensor signal is transmitted, interpreted and may be acted upon either by an automated process, or by individuals monitoring the sensor. There are two major definitions of the term “biosensor”. A biosensor can reasonably be defined as a device that detects biological organisms, or some signature of the presence or status of biological organisms (<https://www.nature.com/subjects/biosensors>). Alternatively, the definition may be expanded to a device that uses some aspect of biology, entire organisms, molecules of biological origin like antibodies, or synthetic molecules having properties similar to biological molecules such as aptamers, and placing the biological components in association with solid state sensor components to generate a signal (<https://www.mdpi.com/journal/biosensors/about>). In this second definition the target of the sensor does not need to be a condition of biological origin. An example of the former case would be a fluorescence detector detecting the change in fluorescence at a given wavelength, and correlating this with the presence of some indicator compound such as chlorophyll or phycocyanin, related to microorganisms of interest, such as cyanobacteria, and perhaps further

relating this to the biomass of the cyanobacteria (Hodges et al. 2018). An example of the latter case might be the use of a microorganism or biochemical process derived from a microorganism to detect changes in a non-biological material of interest, such as a sensor that uses the binding properties of artificial DNA to detect heavy metal ions in water (Lai et al. 2019).

Note that these competing definitions of biosensor, while different in implication, are not mutually exclusive and have significant potential overlap. Presumably then, a microbial biosensor could be any of the possible combinations. These could include a non-biological sensor that detects microorganisms, a sensor that uses whole microorganisms, or other biomolecules derived from microorganisms to detect some non-biological environmental condition. A biosensor could also be a sensor which uses microbiologically derived biomolecules or microorganisms to detect the presence or some signature of microorganisms.

Given that a sensor element is something that reacts to changes in the environment, then the results of a survey of environmental conditions undertaken using traditional techniques also may be considered a sensor as long as the parameters being measured are sensing an underlying condition (Gordon et al. 2018). This definition is probably less helpful when considering the future of sensor technology. It may be useful to consider other parameters in the definition of a sensor. For example, a lateral flow chromatography test for the detection of microorganisms (Biagini et al. 2006; Bu et al. 2019), could be considered a “microbial biosensor” as defined, particularly if it detects the target

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using an antibody/antigen interaction. However, this technology is perhaps more frequently described as an assay, rather than a sensor. The distinction may be that an assay requires separate sample collection and analytical steps, which may be conducted individually or in batches. While these technologies may be useful in environmental detection programs, sensors that reduce the delay between sampling and result, directly providing results through automation, open new possibilities for use in environmental applications. In this example, the parameters of time, and automation are significant. A device that collects a sample, and conducts a lateral flow chromatography assay, and transmits the results of the assay would be closer to the definition of a sensor. While a sensor does not necessarily require full automation, elements of automation are typically incorporated into sensors. Automation of sensor detection, analysis, and information transmission reduces the time between a change in the environment, and the availability of that information.

There are many types of biological materials, or materials that mimic biomolecules which have been used in biosensors. The lock and key type relationship of antibody/antigen capture has proven very useful in sensor technology. These types of systems often use antibodies bound to a substrate, to capture microbial antigens. The antigen may be an entire microorganism, such as a *Salmonella* spp. bacterium (Farka et al. 2016), or a metabolic product characteristic of a targeted microorganism (Wong et al. 2019). Another biomaterial used to detect specific microorganisms using the lock and key type strategy are nucleic acid oligonucleotide probes called aptamers. Aptamers are frequently artificially selected for their ligand-like capture of specific targets through selected evolution of ligands by exponential enrichment (SELEX). These have shown some success in detecting complex microbial organisms in clinical samples (Hu et al. 2018), and have shown potential in environmental matrices (Weerathunge et al. 2019). The oligonucleotide does not need to be DNA. An example of a molecule that mimics properties of a biological molecule is the peptide nucleic acid (PNA). PNA's have a peptide backbone and nitrogen bases. Single stranded PNA molecules may serve as aptamers, or as hybridization partners for nucleic acid molecules. These have been used in the detection of *Escherichia coli* ribosomal RNA (Zhang et al. 2018). PNA materials are more environmentally robust than DNA and may lend themselves to use in applications where temperature, and other conditions are not conducive to the use of DNA probes.

Biosensors designed for the detection of nucleic acids or a targeted organism in a sample present an opportunity for increasing the sensitivity of detection using well-known chemistries. Although theoretically small copy numbers of target oligonucleotides may be detected from relatively unprocessed samples, often a few cycles of a replication through either the PCR reaction (Liu et al. 2018), or an isothermal nucleic acid amplification technique such as NASBA (nucleic acid sequence-based amplification) (Won and Min 2010), or LAMP (loop-mediated isothermal amplification) (Priye et al. 2018) may be used to increase the number of targeted oligonucleotides to be detected. The amplified targets may then be hybridized or captured by a capture oligonucleotide. This capture oligonucleotide may be in suspension, or bound to a substrate. If in suspension, it could be captured on, or bound to a substrate for ease of measurement at a subsequent step. These substrates may be a membrane, paramagnetic bead, microelectronic feature, light channel, manufactured surface or other novel solid-state substrate. The capture molecule is exposed to the sample, containing the target of interest. The capture reaction is allowed to occur, and may require sample manipulation, such as optimal temperature or salt concentrations. Once the target is bound to the capture molecule, the target and capture molecule complex may be detected directly, or may be exposed to a reporter molecule. Direct detection may measure a change in the conductivity or resistance of a nanowire, a change in the mass of a microcantilever device, a change in the optical properties of the substrate, for example a change in the spectral qualities of light incident on the substrate. The use of a second reporter molecule is

called a “sandwich” type assay. A sandwich assay uses a reporter molecule that binds to the target of interest. These reporter molecules may be antibodies, aptamers, or other novel molecules, and it is not necessary that the capture, and reporter molecules be the same class or type of molecule. The reporter molecule typically has a domain containing a moiety selected to enhance detection in the system used. This moiety may be an enzyme, fluorophore, colored bead, or other convenient detector. The assay is called a sandwich assay, because the target is sandwiched between the capture molecule and the reporter. The concentration of the target in the sample may then be related to the intensity of the signal from the reporter as measured by the signal detection system, or through the kinetics of the reaction. The specificity of the system may be enhanced by using two molecules that are both specific for the target, however, it is also possible to enhance the sensitivity of the system through judicious use of semi-selective molecules.

All techniques to detect genetic material require that the genetic material be presented to the sensor. For detection of specific organisms, this generally requires disruption of the cell. Alternatively, the technique may rely on DNA present in the environment (eDNA) which may have been shed by organisms either through cell death, or other mechanisms. The CRISPER-Cas technology, for example, is being adapted for use in environmental detection, of eDNA (Williams et al. 2019). Depending on the goals of the program where this is being used, the relationship between eDNA and the presence of specific organisms of interest may need to be established in the body of water of interest.

While many biosensors use biological molecules combined with solid state or selective membrane sensor elements to detect targeted molecules in the sample, some biosensors use entire organisms or cells for the sensor element monitored by solid state or photonic based technologies. Some prominent examples of these use genetically engineered cells to detect the presence of biotoxins in the environment (Banerjee et al., 2013). Naturally occurring whole organisms such as luminescent bacteria have been used for the detection of general toxicity (Woutersen et al. 2017). There are examples of whole cell biosensors detecting microorganisms, including a sensor which detects viruses and virus proteins (Guerreiro et al. 2019; Kintzios et al. 2004). Whole multicellular organisms such as *Daphnia magna* have been used for toxicity detection as well (Huang et al. 2017). Some more complex devices may condition the water to ensure that temperature fluctuations, or other water parameters, do not influence the biological responses monitored by the sensor.

Signal to noise ratio is a problem in sensor development. In environmental samples, there are frequently several steps in sampling and analytical methods designed to boost the signal to noise ratio. Sample processing methods are occasionally implemented to reduce the relative impact of “noise” in environmental measurements, but this often adds significant time and complexity to environmental measurements. The alternative has been to attempt to boost the detected signal with increasingly sensitive sensor devices. The relatively small signals presented by low numbers of environmental targets being detected may be measured by the deflection of a very sensitive microcantilever (Liu et al. 2019), which detects the change in mass of a surface functionalized with antibodies, when an antigen binds to the antibody. Antibody antigen interaction may also be detected by detecting an alteration in the properties of an evanescent wave, due to the proximity of the antigen to a wave guide, following the antigen antibody interaction of an antibody functionalized wave guide (Taitt et al. 2016). Detection of DNA have been reported by techniques such as by monitoring the conductivity of nanowires (He et al. 2008) to which detection oligonucleotides are bound, and monitoring the change in charge potential following binding of the DNA target.

An alternative for, or adjunct to highly sensitive detection techniques is the use of sample processing. The idea of sample processing is to increase the likelihood of interaction of the target of interest with the detection element. This may be done by removing the water while retaining the targets, through filtration, or by increasing the number of

targets in the sample through cell culture (Angelescu et al. 2019), DNA amplification technologies such as PCR (Liu et al. 2018), or through other means.

Microorganism sensors are constrained by the relative abundance of the organisms, and if they are not targeting entire organisms, the quantitative efficacy of the technique may be in question. The presence of a signature of a microorganism may depend on the length of time since the signature was produced, the route through which the signature entered the environment, the lifecycle stage and population health of the organism, or many other factors. The signatures of some organisms may persist beyond the time when the organism presents a public health risk or environmental threat, or that relationship may be non-linear. In these cases, the comparability of this data with existing risk related techniques, and the ultimate purpose of the sensor must be considered.

An operational problem with biosensors and any sensor in general, is the lifespan of the sensor element. Lifespan includes the shelf stability of the sensor element, along with relative lifespan during deployments in various types of water. Fouling can reduce the useful life of a sensor, and sensor life may be less than 24 h (Woutersen et al. 2017). Some biological materials have relatively narrow ranges of environmental tolerance. Biological molecules used on sensors may be intolerant to heat, cold or sudden changes in temperature, and conductivity, salinity or many other factors may alter their performance. The use of guards and copper components in sensor and sensor housing design may reduce biofouling but may also contribute metallo-ions which can have an impact on the assay result depending on the mechanism of the sensor element. It is difficult to quantify the effect of fouling on sensor performance (Samuelsson et al. 2018). Fouling and limited sensor life should be counteracted by the development of effective quality assurance protocols to ensure that data account for these adverse effects, that the effects may be recognized, and remediated effectively. The associated costs of fouling and sensor element lifecycle must also be considered in the implementation of any biosensor-based program.

Biosensors may have a distinct advantage over other methods for the analysis of microorganisms. Some microorganisms are defined by their antigenic properties, or genetic identity. Hence, biosensors using these technologies for detection provide a direct link to the identification of an organism. Other biomolecules, may also be identified by their antigenicity, such as the use of enzyme linked immunosorbent assay (ELISA) in the identification of algal toxins (Yeager and Carpenter 2019). Users must be sensitive to the value of information when interpreting the information provided by the sensor.

Sensors are typically a mechanism to collect more data than would normally be available by sampling and analysis. Sensors may also provide a mechanism to collect information at times and in places where sampling and analysis are inhibited due to cost or other factors. Areas where the most benefit may be derived from microbial biosensors are determined from the parameters of a project plan that describes: the underlying question to be answered, the types, methods of analysis and interpretation of data collected. Answering a question concerning the impact of rainfall on contamination may require a different set of data inputs than answering a similar question concerning whether, having removed a source of contamination, the body of water is recovering. There are plausible applications for biosensors to answer many questions, and in some cases, they may be the preferred solution.

One hindrance to the adoption of biosensors is the lack of a specific mandate for their use. The microbiological quality of source water is generally not continually monitored. There are locations with monitoring criteria for the microbiological quality of recreational water that were created within the context of the Clean Water Act (CWA) (Guerreiro et al. 2019). Current regulation for microbial water quality for recreational waters focuses primarily on the fecal indicator bacteria, culturable enterococci, and *Escherichia coli* (U.S. EPA, 2012). These criteria also consider the potential for additional methods, and additional microorganisms, such as *Bacteroides* spp. *Clostridium perfringens*,

human enteric viruses, and coliphages. During a five-year review of these criteria (U.S. EPA, 2018), cyanobacterial toxins were included as significant potential health risks, but no single criterion was established at that time for all bodies of water. This report noted that there was insufficient evidence to correlate cyanobacterial cell counts with health outcomes. States like Ohio have responded to this regulatory framework by adopting strategies (Ohio Department of Health et al., 2016), conducting monitoring and creating public notification frameworks (Ohio Department of Health 2019). Microorganism contamination standards for surface waters thus typically focus on indicator microorganisms, or algal populations for the protection of recreational exposure to water. If sensor output is correlated with data types and quality provided by the methods currently in use, sensors could be adopted as more cost-effective ways of ensuring public health in this regulatory framework. As an alternative, sensor data could be used in conjunction with public health studies to develop affirmative evidence of a correlation of some sensor output, or pattern of sensor data that may be protective of public health.

Another alternative to regulatory compliance-based monitoring is the systems idea embodied in EPA's Water Security Initiative Contaminate Warning System (U.S. EPA, 2008). One of the main concepts of this system was to determine the normal background level of each parameter being measured, to determine when a deviation from that background is enough to indicate the presence of a contamination event. In this way contaminants may be detected in the absence of specific contaminant detecting sensors. This strategy is a mechanism for overcoming the lack of contaminant specific detectors, as well as facilitating recognition of changes in a normal or baseline conditions, which could lead to a more thorough investigation to identify a specific contaminant, or condition leading to contamination. These types of baselines may not be possible to establish in environmental waters, or the establishment of a baseline may be so costly and time consuming that continuous efforts in monitoring a baseline set of conditions in environmental water becomes unjustified.

There are few specific monitoring criteria for the continuous monitoring of source water to be treated for consumption. Strategically, source water is initially surveyed for the level of contamination, and treatment processes optimized or implemented to manage the associated risks. Following the initial survey, there is typically no continuing requirement for monitoring of source water for drinking water treatment. An exception to this would be if there were additional risks to public health detected, and these risks are not effectively managed by existing systems. Public health is protected from potential microbial contamination of surface water through a series of drinking water treatment technologies providing multiple barriers to the contamination of drinking water and have been demonstrated efficacious to the known microbial public health threats.

Outside this risk management process, there are circumstances where information concerning microbial contamination could be used to influence the operation of water treatment for drinking water. Potential applications of this may be for detection of high numbers of toxic cyanobacteria, or the presence of cyanotoxins, or other microbial threats at levels of concern in the source water. In these cases, it may be possible for a water treatment system to adjust its processes to manage this risk appropriately in a cost-effective manner that is protective of public health given timely information on the occurrence of the threat in source water. One mechanism for this would be linking sensor input directly with supervisory control and data acquisition (SCADA) systems to directly respond to changing conditions without human intervention, but with a human veto possible. An additional potential use case for monitoring source water for a water treatment authority is for the purpose of long-term planning. Continuous monitoring should show trends in water quality which may necessitate changes in treatment or may provide opportunities to optimize drinking water treatment options in the presence of changing source water conditions. In cases where there are multiple potential sources, biosensors may permit

optimization of water source use, and resource optimization may be enhanced by additional data on source water quality. Sharing data from source water quality monitoring with recreational water decision makers may help bolster the case for improved watershed management, and increase the utility of the data, potentially permitting a splitting of costs.

It may be easier to adopt sensors as replacements to traditional monitoring methods if they use the same underlying technology. One solution to this has been to automate the sample collection and culture analysis using versions of standardized methods into a field portable unit (Angelescu et al. 2019). Also, when current techniques rely upon the detection of genetic material through PCR, sensors which rely upon PCR in the detection, or sensors which detect genetic elements in a sample using alternative techniques could reasonably be alternatives to other PCR assay techniques. Other sensor technologies, such as antibody/antigen-based capture techniques may face greater challenges in demonstrating data comparability with culture or PCR based techniques currently used for monitoring.

The lack of data comparability is compounded by the inherent nature of most sensors. Since many sensors can collect data more rapidly than sampling and analytical methods, the additional data poses a conundrum. If there is a regulatory requirement for a sampling schedule, say a single sample per day, or week, then devices which collect data more frequently may pose problems. Collecting more data than is necessary may result in a greater number of exceedances than had been previously reported. These exceedances may not be due to degraded water quality, but rather to conducting measurements at times and frequencies that were not anticipated in the initial implementation of the monitoring program. For instance, there may be a requirement to collect a sample at a specific time, yet samples collected and analyzed outside this time window may not have the same relationship to a public health threat and may unnecessarily raise concerns, or may demonstrate an insufficiency of the current methods to protect public health.

Correlating sensor data with public health survey data is another potential pathway to adoption of novel sensor technology in surface water monitoring. Health studies are expensive, and difficult to undertake. Health surveys resulting in a quantitative microbial risk assessment generally require many years and involve multiple locations and substantial organizational support (DeFlorio-Barker et al. 2018). Attribution of significant health effects to recreational exposure to surface water and correlation of these results with biosensor output will likely require an even more extensive effort than studies used for the current regulatory framework.

Sensor technology that automates the PCR assay process may be one condition under which sensors are employed in recreational water management. Application of any sensor technology requires consideration of sample frequency and signal quality. Long term assessment of sensor performance including adequate measures of false positive rates should be included in all biosensor-based monitoring programs. False positive and false negative results are inherent in all assay techniques. Without a plan to manage potential false positives, there may be occasions when incorrect decisions are made that have multiple adverse outcomes, including economic burden, and loss of confidence in a monitoring program. Since false negative results lead to inaction, but this can be overcome by taking additional measures or samples. A subsequent measurement provides another opportunity for correcting the false negative. Thus, from a programmatic standpoint, false negative rates are not considered as problematic as false positives. Monitoring more frequently does not necessarily provide for a better information stream. If a decision algorithm is based on a given rate of environmental quality information, increasing the frequency at which this information is collected or provided may require revisiting the decision algorithm to ensure that it is effective, and not overly restrictive.

Whether in the context of replacing required monitoring activities,

or supplementing environmental information for other decision-making purposes, the operational considerations of a sensor is another critical element in developing the requirements for a sensor. Plans for an extended autonomous deployment will require different performance features from a sensor than a plan to carry a sensor to multiple locations in the field by a data collector. Sensors should be chosen to satisfy operational characteristics. For example, some sensors may require anti-fouling measures, or some sensors may not be amenable to remote telemetry of data. Longer unattended deployments are frequently more taxing and difficult to support, and more likely to experience problems of data quality and operational failures. Biosensors designed for long term deployment in the field must be ruggedized against field conditions and configured to collect data in adverse and changing environments.

An additional way that sensor technologies may demonstrate utility is in facilitating citizen science. One of the underpinning philosophies behind environmental protection is making information known to the public. Citizens, given accurate information can make difficult choices to balance the equities concerned in complex environmental problems. This concept is embodied in the National Environmental Policy Act of 1969 (USC 1969), and is evidenced by organizations and individuals who conduct measurement and analysis of water samples from their local waterways and provide input into decision making processes at public hearings throughout the nation. Data from monitoring is made available at a number of websites, for example the wiki watershed (<https://wikiwatershed.org/monitor/>) and others. Beyond this, these efforts are supplemented by a growing “do it yourself” (DIY) movement. There are user communities of interested individuals interested in creating devices to monitor environmental conditions in their local area. Highly valued characteristics of sensors for this community are ease of use and calibration, stability of operations, capability for extended deployments, low lifecycle cost, low initial cost, and robustness in adverse environments.

The Open Source, DIY, and low-cost sensor development communities have been supportive of individuals, and small organizations, as well as larger academic, and even governmental efforts to provide water quality monitoring and to make this data available to the public. DIY sensor projects may be an effective way to engage citizen science communities and may encourage a more thorough understanding of the technology being used, the environmental parameters being measured with regards to their interaction with the technology, and may also facilitate maintenance, and quality assurance. An enduring concern of these efforts is the quality assurance of these data (Kosmala et al. 2016). A sensor that is fouled, suffers a communication failure, has been incorrectly calibrated, or is otherwise providing faulty or unreliable data may lead to incorrect conclusions being drawn from the analysis of that data. There are efforts ongoing by a number of citizen science oriented groups to incorporate principles of quality assurance into the collection of citizen science data. There are organizations which aim to ensure that citizen scientists are trained in the appropriate use of their equipment, sample collection and preservation techniques, calibration and installation of monitoring equipment. The citizen scientists are then encouraged to adopt a quality assurance project plan (QAPP) (Barzyk et al. 2018) and provided with a manageable template for this (U.S. EPA, 2019). In some cases, organizational support may be provided for independent review of QAPPs and for ongoing training and quality assurance monitoring. There are also organizations dedicated to providing data repositories, some of which include mapping and analytical tools.

It is not the purpose of the description of the potential for biosensor use by citizen scientists to provide a comprehensive discussion of citizen science. Links to citizen science resources may be outdated, misleading or not representative of the support available for any specific citizen scientist. Individuals interested in citizen science represent a significant potential outlet for the advantages of microbial biosensors and potential for automated data collection. The possibility of high

sensitivity and specificity of biosensors, and the ability to target microorganisms of interest in a watershed, make these tools especially well-suited for citizen science applications. Factors that must be addressed by technology developers for greater inclusion into citizen science effort include cost, reliability, ease of use, data interpretation, quality assurance, and possibly provision of support for programmatic implementation of the technology by citizen scientists, who may be loosely organized groups of individuals.

One theoretical consideration that may limit the use of biosensors is the limit of detection. The limit of detection of biosensors for microorganisms must be close to the limits of detection of other methods for these devices to be useful adjuncts to monitoring programs. In practice, however, there will be some organizations that will ignore this consideration, given a general knowledge of the presumed limit of detection of the sensor. In this case, if there is some general understanding of the limit of detection, even if the sensor is relatively insensitive compared to routine laboratory testing, the sensor will serve to detect “breakthroughs” or large events where additional sampling and analysis may be warranted. If the sensor is relatively more sensitive than selected analytical methods, there is the potential for misinterpretation of data. Thresholds for analytical detection which provide a level of public safety do not necessarily need to be adjusted simply because a more sensitive method or sensor is developed, rather the method or sensor may serve as a monitor for background levels of organisms of interest. A sensor may also serve as a *de facto* limit of concern, but this may result in dissatisfaction with the results, if collected samples are mismatched with the results of the sensor. Rather than being interpreted within the performance characteristics of each technique, the idea of false positive and negative will certainly be introduced into the discussion, and it may be difficult to select an appropriate gold standard method against which to compare methods. The ultimate determination of whether any sensor or method will provide actionable data will rely upon the goals and objectives for the project, and the use of techniques that will provide appropriate quality of data (U.S. EPA, 2006).

Sensor performance is often measured by receiver operating characteristic curves (Hubaux and Vos, 1970), or through a method detection limit (CFR 2017) although this may be less commonly used for evaluating biological detection techniques (Humrighouse et al. 2015). Sensors may also be described in terms of their sensitivity, specificity, quantitative accuracy, minimum detection limit, precision and accuracy. Finally, “fit for purpose” may be used as a criterion for sensor testing. The appropriate sensor characteristics should be chosen to match with the predetermined data quality objectives for the program.

In cases where more data is better than less data for making decisions, sensors are typically a mechanism to collect more data than would normally be available. Many sensors can operate in unattended deployments and to transmit data from remote locations. In many cases water quality changes rapidly during periods of high flow: rainfall, ice off or floods. To capture these changes a programmatic plan may require sample collectors to travel long distances with little advanced notice, at any time during the day or night, during inclement conditions which may include travel through dangerous storms or flood waters. An unattended sensor deployment and remote data transmission could reduce or eliminate the need for this activity. Using a biosensor in these circumstances may permit data to be coupled with hydrological or meteorological data for a more complete data set.

Microbial biosensors are currently available in the commercial marketplace and will rapidly increase in prevalence. The use cases for this technology are only now being understood and developed. The technology has so many advantages over traditional sampling and analysis, that it is difficult to envision a future where this technology is absent. As with every technology, there will be a transition period when the older technologies are displaced, and more effective operational guidance is developed to capitalize on the advantages of the newer technology. The nearly instant data communication and networking potential of sensors alone will be a revolution in the awareness of

environmental conditions. Currently the reductions in cost of telecommunications technology are enabling mesh networks of sensors to reduce the cost of deployment and increase the information available about water. Once implemented, there will be massive new datasets to probe and analyze, and more complex questions can be asked and answered. The beginnings of this are evident where water sensors are being deployed to measure physicochemical parameters. A more fully integrated understanding of the microbiota will lead to a greater understanding of the health of waterways and better tools for addressing environmental degradation, as long-term strains on water resources and water quality increase. These tools will permit more efficient application of costly interventions and permit more rapid and effective assessment of the efficacy of efforts to improve water quality.

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