Adv Biochem Engin/Biotechnol (2009) 116: 99–123
DOI: 10.1007/10_2008_51

Published online: 21 May 2009

Environmental Applications of Photoluminescence-Based Biosensors

Kenneth F. Reardon, Zhong Zhong, and Kevin L. Lear

Abstract For monitoring and treatment of soil and water, environmental scientists and engineers require measurements of the concentration of chemical contaminants. Although laboratory-based methods relying on gas or liquid chromatography can yield very accurate measurements, they are also complex, time consuming, expensive, and require sample pretreatment. Furthermore, they are not readily adapted for in situ measurements.

Sensors are devices that can provide continuous, in situ measurements, ideally without the addition of reagents. A biosensor incorporates a biological component coupled to a transducer, which translates the interaction between the analyte and the biocomponent into a signal that can be processed and reported. A wide range of transducers have been employed in biosensors, the most common of which are electrochemical and optical. In this contribution, we focus on photoluminescence-based biosensors of potential use in the applications described above.

Following a review of photoluminescence and a discussion of the optoelectronic hardware part of these biosensor systems, we provide explanations and examples of optical biosensors for specific chemical groups: hydrocarbons and alcohols, halogenated organics, nitro-, phospho-, sulfo-, and other substituted organics, and metals and other inorganics. We also describe approaches that have been taken to describe chemical mixtures as a whole (biological oxygen demand and toxicity) since most environmental samples contain mixtures of unknown (and changing)

K.F. Reardon (✉) and Z. Zhong
Department of Chemical and Biological Engineering, Colorado State University,
Fort Collins, CO, USA
e-mail: kenneth.reardon@colostate.edu

K.L. Lear
Department of Electrical and Computer Engineering, Colorado State University,
Fort Collins, CO, USA

composition. Finally, we end with some thoughts on future research directions that are necessary to achieve the full potential of environmental biosensors.

Keywords Antibody • Biosensors • Enzyme • Induction

1 Introduction

1.1 Photoluminescence-Based Biosensors

Environmental scientists and engineers frequently desire to measure the concentration of chemical contaminants in soil and water samples. Although laboratory-based methods relying on gas or liquid chromatography can yield very accurate measurements, they are also complex, time consuming, require sample pretreatment, and use expensive equipment. Furthermore, obtaining measurements directly at the source of the sample is often important, but modifying chromatographic methods for field use has proven to be difficult.

To overcome these limitations, researchers have developed a variety of assays and sensors for environmental contaminants. Immunoassays have been developed for field use, particularly for larger analytes such as polycyclic aromatic hydrocarbons [1] and chlorinated pesticides [2]. However, these assays provide only a single measurement each, cannot be used in situ (and thus are subject to sampling errors), require reagents and several analytical steps, and suffer from poor specificity for smaller molecules.

Sensors are devices that can provide continuous, in situ measurements, ideally without the addition of reagents. A biosensor is a device in which a biological component (typically an enzyme or antibody) is coupled to a transducer, which essentially translates the interaction between the analyte and the biocomponent into a signal that can be processed and reported. A wide range of transducers have been employed in biosensors, the most common of which are electrochemical and optical.

In this review, we focus on biosensors in which photoluminesence serves as the transduction mechanism. Optical biosensors in general have advantages over those relying on other transduction methods. These desirable characteristics include small size, the ability to measure over long distances with minimal signal loss, and no requirement for reference cells/sensors. In addition, photoluminescence-based biosensors have the ability to achieve higher sensitivity with lower interference from other sample components than is the case with absorbance or other types of optical biosensors. Photoluminesence transducers can be coupled to a variety of biocomponents, including enzymes (purified and in whole cells), antibodies, aptamers, and whole cells and tissues. The majority of reported photoluminescence-based biosensors have used enzymes. Antibodies are more commonly used in bioassays because of the need in most detection formats to add reagents and wash the reactive surface, but a few biosensors have been described in which these steps have been modified to allow semicontinuous measurements.

1.2 Environmental Applications for Biosensors

The range of application scenarios for biosensors in environmental science and engineering is broad, but in general two categories of measurement goals can be proposed. First, measurements may be made initially to assess and characterize the types and levels of contaminants at a particular site or in a body of water. In this case, only one time point may be desired (although different locations are normally evaluated), and often there is little previous information about the contaminants at the site. The second goal is monitoring. In this case, the objective is to track the concentration of known contaminants over time at one or more defined locations. To date, these monitoring measurements have consisted of a series of discrete measurements (assays). However, biosensors, with their ability to provide continuous, or high frequency semicontinuous, measurements, offer the potential for more information about these contaminant levels.

Examples of some application scenarios are:

- Water treatment process monitoring. In both drinking water and wastewater treatment processes it is desirable to monitor contaminant levels for the protection of human and environmental health. Given the very high flow rates of these processes, continuous monitoring of specific chemicals would be desirable; however, but this is not possible with currently available technology.
- Protection from chemical terrorism of water supplies. The possibility of terrorist attacks by the addition of chemicals to water supplies has arisen in recent years.

Low cost, continuous measurements of specific chemicals or of toxicity would protect people from this danger.

- Characterization of contaminated sites. When soil and/or groundwater contamination is suspected at a site, the type and level of contaminants at the site must be profiled so that an effective remediation strategy can be developed. Using laboratory-based measurements requires the removal of samples and a waiting period before data are obtained, and then several additional rounds of sampling and analysis are usually needed. The use of biosensors for this initial assessment phase would enable the site to be completely characterized in one sweep, potentially saving both time and money.
- Monitoring of remediation processes. Once a remediation process has been designed and implemented at a contaminated site, its effectiveness must be established through a program of periodic monitoring. This could be performed with biosensors on site, either in the assay mode or as part of a continuous monitoring system.
- Environmental monitoring. It is often desirable to monitor sensitive water sources (ground water wells, rivers, lakes, etc.) that are downgradient from industrial sites and other sources of contaminants. Traditional manual sampling and laboratory analysis are too expensive and too slow to accomplish this monitoring goal, but biosensors that can measure continuously and in situ could play an important role in this application.
- Precision agriculture. The goal of precision agriculture is to apply the correct amount of fertilizer and pesticide on every portion of a field, recognizing that different amounts are required depending on slope, exposure, soil type, and other factors. The use of in situ biosensors would provide feedback to farmers about these chemical application rates.

In all of these scenarios, biosensors could provide important information not readily available using traditional analytical technologies, particularly when continuous or in situ measurements are desired. Biosensor and traditional technologies could also be paired, with biosensors used on site for immediate data acquisition and traditional measurements performed later in a laboratory with high accuracy methods.

1.3 Scope

Several general reviews on biosensors for environmental applications were published in the 1990s [3, 4]. In this contribution, we focus on photoluminesence-based biosensors of potential use in the applications described above. In general, only reports of biosensors are included, although some assays are mentioned when it is evident that the underlying measurement technology could be modified to yield continuous, in situ data. In addition to chemical-specific biosensors, we consider those that report an assessment of toxicity.

2 Characteristics and Challenges of Environmental Measurements

Analyte concentration measurement in any practical application faces numerous challenges; for example, sensors must be robust, free of interferences from chemical, physical, or biological factors, and retain activity for a long period. However, measurements in surface or ground water matrices have additional attributes that provide challenges for biosensor development, especially in the case of in situ measurements. Some of these are:

- Wide range of analytes. Contaminated ground water, waste water, and other environmental samples usually contain many different analytes of interest.
- Low analyte concentrations. In contrast to analytes in industrial bioprocesses, in which the relevant concentration range is usually in the mgL^{-1} to gL^{-1} range, concentrations of interest in environmental samples are dictated by governmental health regulations and are typically around 1 $\mu g\ L^{-1}$ or less. Traditional measurement methods rely upon a concentration step to achieve these low limits of quantitation, but biosensors must be capable of high sensitivity without sample pretreatment.
- Complex matrices. Environmental samples are frequently complex in chemical, biological, and/or physical terms. The presence of soil particles, soil organic matter, microorganisms, salts, nonanalyte organics and metals, and other factors are challenging for biosensor development, and the range of these factors in different samples makes the development of robust calibration protocols a vital consideration.
- Demands of field applications. Equipment intended for use at a site (rather than in a laboratory or process environment) must be durable and not shock sensitive, must not require an external power source, and must not be affected by heat, cold, direct sunlight, wind, and other factors.
- Expectations for low cost. Because environmental measurements are performed in a regulation-driven context and not a profit-driven one, measurements and measurement equipment are expected to be inexpensive and to have a long life.

3 Optoelectronic Hardware

3.1 Optical Biosensors

Optical sensing encompasses an enormous field of technology with applications spanning from imaging to spectroscopy on scales from astronomical ranging to subwavelength interferometry. At the core of optical sensing is metrology of one of the fundamental parameters of electromagnetic waves: phase, polarization, or most commonly, amplitude. With reference to Fig. 1, amplitude measurements relevant

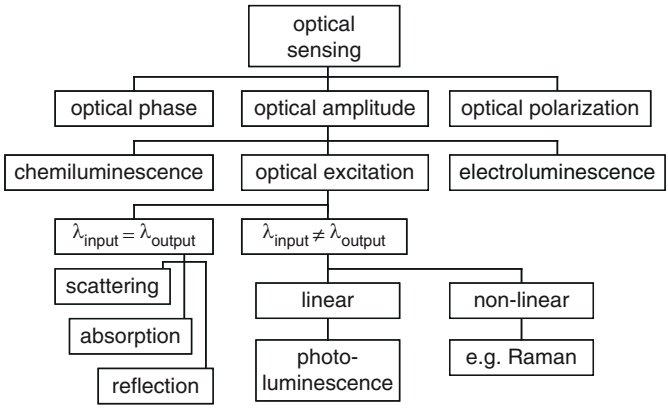

Fig. 1 Classifications of different optical sensing approaches. Photoluminescence is the mechanism used for environmental biosensors discussed in this work

to environmental sensing can be further narrowed based on the energy source used to create the optical signal to be monitored. By far, the most widely practiced approach is the delivery of optical energy to produce the output light. Conventional absorption, reflection, or scattering measurements infer information from changes in the probe light itself after interaction with a sample. Another class of optical sensing relies on optical emission from the sample at a different wavelength than the input light. The wavelength shift can either be a result of nonlinear mixing of the light with itself as in harmonic generation or with other oscillations such as vibrational states giving rise to Raman shifts or as a result of the electronic excitation of an atomic or molecular system that releases a portion of the excitation energy as a photon of another color. The last process is termed photoluminescence (PL) and includes two phenomena, fluorescence and phosphorescence, that differ in the details of quantum transitions but are quite similar in practical application. Fluorescence occurs when an optical transition is highly probable resulting in the rapid production of a photon. Phosphorescence occurs in the case of nearly forbidden transitions that allow the molecule to maintain the excited state for much longer times before relaxing via photon emission, typically with a greater difference in energy between emitted and excitation photons than in the case of fluorescence. A formal distinction between fluorescence and phosphorescence divides the two at a decay lifetime of 10 ns with fluorescence lifetimes being shorter and phosphorescence lifetimes being longer.

Photoluminescence may be employed for sensing by exciting a sample and directly looking for the PL spectrum of the analyte or by indirectly observing changes in the PL of another species affected by the analyte. Optical enzymatic biosensors typically use an indirect mechanism whereby the products of the reaction modify the PL efficiency of nearby dye molecules. For example, conversion of toluene by a monooxygenase consumes dissolved oxygen in the proximity of an oxygen-sensitive ruthenium-based dye altering the dye's PL efficiency. Higher analyte levels result in higher reaction rates and thus depleted oxygen levels, reducing oxygen quenching

of the ruthenium dye and increasing the associated phosphorescence emission under constant excitation power.

3.2 Instrumentation Components

A variety of optoelectronic options are available for sources and detectors to provide an interface between electronic power supplies and signal processing and the optical realm in which PL sensing occurs. In conventional linear photoluminescence (as opposed to two-photon excited PL), the optical source must deliver a sufficient amount of excitation power in the absorption band of the photoluminescent material, which will be at higher energy and thus shorter wavelength than the material's emission peak. Most fluorescent and phosphorescent dyes have been developed to emit in the visible spectrum due to the limited response of conventional photomultiplier cathode materials, e.g., S-1, at longer, infrared wavelengths. Thus PL sources emitting in the blue to ultraviolet portion of the spectrum are appropriate for many dyes. Efficient, durable, and compact modern sources in this range are typically light-emitting diodes (LEDs) and laser diodes based on GaN and related III–V semiconductors, although SiC LEDs and halogen lamps were designed into earlier systems. Compared to LEDs, laser diodes provide much greater energy efficiency, higher power, more intense and readily focused output beams, an output spectrum less than 1 nm wide, and the potential for polarized operation. Laboratory-grade blue laser diodes are still 10–100 times more expensive than comparable red laser diodes, but spin-off technology from Blu-ray disc players should virtually eliminate this difference within a few years. Lower cost is the major advantage of blue LEDs with instrumentation grade devices in molded plastic housings that offer direct fiber connections selling on the order of US$10 in small volumes, slightly less than the cost of laboratory red laser diodes. Direct fiber coupling of LEDs, practically eliminates the advantages of laser diode beam properties relative to LEDs and dye photobleaching at high optical intensities [5] can restrict the ability to fully leverage the higher output powers of laser diodes. With proper drive electronics, LEDs can operate at or near the shot noise limit, while laser diodes frequently exhibit noise at least ten times the shot noise limit although designers should recall that the shot noise limited electrical signal-to-noise ratio is proportional to optical power. The output spectrum of many blue LEDs is approximately 20 nm full-width at half-maximum and contains long tails often necessitating the use of a source bandpass filter to block excitation light falling in the PL emission band. The ratio of peak-to-tail power from laser diodes at high drive currents is much better than LEDs, but the corresponding high power may require a neutral density filter or other component to attenuate the total power.

Akin to the replacement of blue lamps with GaN diodes, advances in semiconductor technology provide alternatives to traditional photomultiplier tubes (PMTs) for sensitive conversion of emitted light into an electronic signal although they are not yet as competitive. While simple silicon photodiodes can efficiently convert incident

photons into electron flow, the resulting photocurrents often fall below the noise floor of subsequent amplification electronics when attempting to detect faint emission from PL. PMTs are advantageous in this situation as their internal gain results in an internally amplified photocurrent above the electronic amplifier noise floor although the internal gain results in a reduced signal-to-noise ratio as does any amplification process. PMT operation in instruments has been significantly simplified through the integration of high voltage generation subsystems so that the instrument only needs to provide standard low voltages, e.g., 12 V, to the PMT module. PMTs are typically the most expensive single component in PL sensor instrumentation with costs on the order of US$1,000. Solid-state replacements for the PMT, avalanche photodiodes (APDs), are becoming more frequently employed, although they do not yet rival PMT performance. Compared to PMTs, typical APDs have somewhat higher noise factors, an order of magnitude higher dark current, and a factor of a thousand or more lower internal gain [6]. APDs are more compact and robust than PMTs. Relatively high prices for APD modules, near those for PMTs, are currently supported by market factors although silicon based APDs should eventually be manufactured at much lower costs than PMTs. Another lower performance solid-state alternative to the PMT is the p–i–n silicon photodiode with hybrid integrated transimpedance amplifier (PIN–TIA) that combines the photodiode and a low noise electronic amplifier in a package size comparable to those used for the photodiode alone. PIN–TIAs require more optical power than APDs and PMTs to produce a signal equivalent to their noise but perform better than discrete integrations of photodiodes with external amplifiers. In low volumes they sell for approximately one-tenth the price of PMTs. For PL sensor systems where low limits of detection are more important than minimal cost or minimal size, PMTs remain the detector of choice for the present.

Beyond the components that convert electrical power to light or light to electrical signals, optical elements such as fibers and filters are required to control optical propagation and block undesired wavelengths. Optical fibers provide a convenient means for transporting excitation light to and returning emitted light from PL sensors. They eliminate the need to maintain optical alignment through much of the system and allow optical sensing at points interior to the analyte volume. Maximization of collected light in PL-based fiber optic sensor systems is paramount and drives the use of fibers with very large core diameters, ~1 mm. Although silica fibers with core diameters ranging from approximately 5 to 50 μm are preferred for most communications applications over distances greater than 10 m, a 1 mm diameter silica fiber would have an impractically large bending radius. Instead, large core plastic fibers manufactured from PMMA are routinely used for sensors. The light acceptance cone, parameterized with the numerical aperture, is usually designed to be much larger in plastic fibers than for glass by using a 5% refractive index step between the core and cladding to achieve numerical apertures as large as 0.5. Plastic fibers are readily available with either SMA- or ST-style terminations where the latter may be preferred due to the presence of an air gap in SMA connections that can result in optical interference. A disadvantage of plastic fibers is their increased absorption over silica fibers. PMMA-based fibers present optical losses

of at least 1.8% and 3.0% per meter in the blue and red portions of the spectrum, and near 620 nm their loss can exceed 10% per meter. However, less common perfluorinated polymer optical fiber has losses below 1.4% per meter for visible wavelengths greater than 600 nm.

Limitations on excitation absorption, quantum efficiency, and light collection often result in the need to detect emitted fluorescence or phosphorescence that is orders of magnitude smaller than scatter and reflections of excitation light requiring spectral filtering to uncover the emitted signal. A long pass or bandpass filter that passes most of the longer wavelength emission while strongly attenuating short wavelength excitation light is frequently employed in front of photoluminescence detectors. Colored glass filters are occasionally used, but thin film interference filters offer steeper changes in transmission to provide better rejection of excitation that is close in wavelength to the desired PL emission. Gratings or complete spectrometers may also be employed to separate excitation and emission light, but appropriate design is required to address complications due to stray light and multiple diffraction orders. Broadband optical sources such as LEDs or lamps require filtering at the source to prevent the undesired light they generate in the emission band that would pass through the detector filter from reaching the detector. Lasers are less likely to require source wavelength filtering, although it can still be beneficial in reducing spontaneous emission from defect states or lower energy cladding materials in laser diodes or unwanted secondary emission lines in gas or rare-earth doped solid-state lasers. Good quality thin-film interference filters are priced in the range of US$100 and above for small volumes.

3.3 Photoluminescence Apparatus

The most rudimentary apparatus for photoluminescence sensing requires only an appropriate optoelectronic source and detector although wavelength selective elements, optical components for manipulating beams, and supporting electronics are incorporated into practical sensor instrumentation. Figure 2a schematically illustrates the simplest system consisting only of an excitation source, the photoluminescent sample, and an optical detector sensitive to the emitted wavelength arranged so as to not intercept reflected or transmitted portions of the pump beam from the source. As mentioned above, practical configurations almost always require an emission band selective filter in front of the detector due to scattered pump beam. One compact arrangement for achieving such filtering while creating a system that can most efficiently collect photoluminescence in a reflection mode is shown in Fig. 2b. Here, a dichroic mirror is employed that transmits nearly all of the outgoing short wavelength excitation from the source while reflecting most of the returning long wavelength emission to the detector. Reflection mode configurations are ideal for single fiber sensors where the excitation and emission light are guided along a common path as seen in Fig. 2b. One advantage of using dichroic mirrors is that the system's optical efficiency can approach unity with almost all of the source

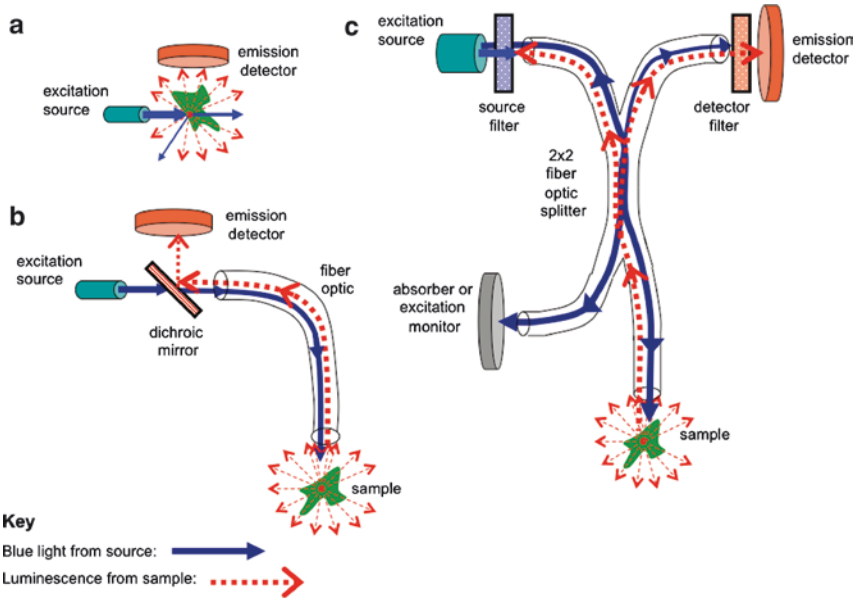

Fig. 2 Configurations for photoluminescence sensing. **a** In the simplest apparatus, light is not confined by fiber optics and the detector is positioned to not receive the transmitted or reflected excitation beam. **b** In a system where the excitation light and emitted photoluminescence travel in a common fiber optic, a dichroic mirror or beamsplitter separates reflected excitation from the emitted wavelengths by only reflecting the latter. **c** An alternative configuration employs bandpass filters and a fiber optic splitter to implement the respective dichroic mirror functions of lightpath combining and wavelength filtering. The leg of the splitter not directed to a sample can be used for monitoring the excitation source or simply dumped to an absorber

light reaching the sample and almost all of the emission light in the fiber reflected to the detector. However, a simple reflection mode fiber system can collect at most ½$(NA)^2$ of the isotropically emitted light at the end of the fiber or less than 13% for a numerical aperture of 0.5. Dichroic mirror designs require at least one lens to focus the source into the fiber optic unless a very narrow divergence laser and large diameter fiber are employed. In either case, the optical source alignment to the fiber through the mirror must be maintained with appropriate tolerances. Large area detectors may relax the alignment tolerances of the return path. The surface quality and cleanliness of the dichroic mirror are critical for effective filtering since scattering from defects or dust on the output side of the dichroic mirror will readily reach the detector.

The complexity of source optics can be relaxed at the cost of system optical throughput in the split fiber system shown in Fig. 2c. Here, a detector filter and, if required, a source filter replace the wavelength filtering functions of the dichroic mirror while the mirror's optical path combining and splitting functions are implemented with a separate 2-input by 2-output, multimode, broadband fiber optic splitter (combiner). One of the splitter inputs is connected to the source while the other serves as a reverse direction output dedicated for the detector. One of the forward direction outputs is usually unused or can provide monitoring of source power, but

cannot be beneficially eliminated as the optics of incoherent multimode fibers result in lost power to this fiber whether or not it is brought outside the fused fiber region inside a splitter. Splitters can be obtained with a variety of splitting ratios with common values of 50/50% or 90/10%. If excess source power is available, then 10% or less of it may be coupled to the sample output leg while 90% or more of the emission light collected in the sample leg is transmitted to the detector. In the case of an LED source where output power does not need to be reduced to avoid photobleaching and the PL is proportional to the excitation power, balanced 50/50% splitting is the optimum choice giving an overall system optical efficiency of 25% as the product of the excitation and emission throughput. This implementation is likely to be no more expensive than the former one based on reduced fixture costs, dichroic mirror costs exceeding those of transmission mode filters, and the modest costs (less than US$100) of fiber splitters. A major advantage of the fiber splitter approach is minimization of mechanical fixturing as fibers can be directly connected to LED sources and many suitable detectors have large sensitive areas providing millimeter-range alignment tolerances.

An alternative to the common 2 × 2 fiber splitter is a bifurcated fiber optic assembly with multiple cores. One configuration has a number of smaller outer cores arranged in a circle about a single, larger inner core for the portion of the fiber cable terminated at the sample. As this cable leaves the sample, it is split or bifurcated into a fiber that only contains the central core that might, for example, connect to the source and another one that only contains the perimeter cores and could connect to the detector. Each core remains isolated from all the other cores throughout the assembly and so all of the coupled source power reaches the sample and all of the collected emission reaches the detector. A disadvantage of this technique is that collection efficiency is reduced by the lack of overlap between the excitation and collected emission regions against the fiber assembly tip. At some distance from the tip, the overlap improves, but here the excitation intensity has diminished and the solid angle subtended by the collection fiber is reduced.

3.4 Multiplexed Fiber Optic Biosensor Systems

As noted above, environmental biosensor systems face the challenge of measuring analytes in matrices with a high probability of interfering signals from other compounds. Even in the unlikely scenario in which interferences from similar molecules are minimal, temperature and pH variations motivate the use of reference sensors. The need to obtain signals from multiple PL sensors in such circumstances prompts the development of multiple channel systems for simultaneously or nearly simultaneously obtaining PL levels from multiple fiber optic sensors. The most straightforward but costly and bulky approach to build multiple sensor systems is depicted in Fig. 3a, where a complete dedicated system including an optoelectronic source (S) and detector (D) and associated filters (F) are connected by a split fiber or equivalent dichroic mirror system to an optode (O) in conjunction with a target enzyme (E1–E3) that is not completely specific. Since the enzymatic sensors can share a

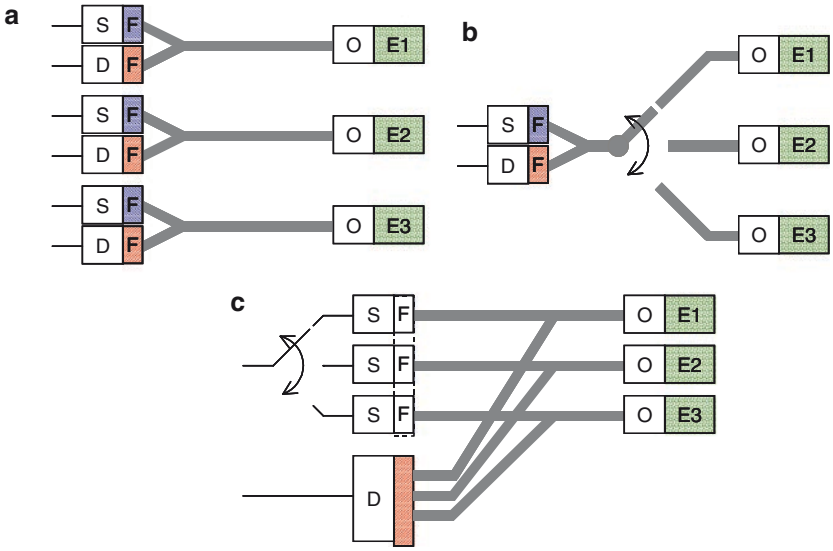

Fig. 3 Multiplexed instrumentation approaches for multiple fiber optic biosensor systems comprised of sources (S), detectors (D), filters (F), optodes (O), enzymes targeting specific compounds (E1–E3), fiber optics (*thick lines*), and electrical connections (*thin lines*). **a** Simple duplication of the full system for each sensor is expensive and bulky. **b** Optical multiplexing offers the smallest component count but relies on expensive custom mechanical switches for plastic optical fibers. **c** Electronic multiplexing requires duplication of lower cost sources and fiber splitters but needs only a single detector and can be implemented with a single source filter and a detector filter. Electronic switching energizes one source at a time

common fluorescent or phosphorescent optode material and structure, the source, detector, filter, and fiber systems for each biosensor can be identical. Further, the rate of change of analyte is slow enough in environmental applications that sampling intervals of several seconds to days are acceptable although the PL measurement requires only 1 s or much less. The low duty cycle and redundancy of hardware motivate multiplexed fiber optic biosensor systems in which a common source and detector are sequentially connected to each biosensor by an automated switching system.

Optical multiplexing via a multipole fiber optic switch as seen in Fig. 3b allows the greatest reduction in total component count. Mechanical fiber optic switches with limited numbers of poles are commercially available as standard products for silica fiber optics intended for communications. However, switches for plastic optical fibers with several poles are only available as custom engineered products with costs that are many times higher than those of PMTs. Hecht and Kolling [7] built a 12-pole fiber optic switch with low insertion loss using two stepper motors and a microcontroller for use in a multiple channel fiber optic sensor system. While they used 100/140-μm diameter silica fiber, the system could be readily adapted to larger diameter plastic optical fibers. The switching time of 20 s was quite slow compared to commercial solenoid driven switches, and mechanical reliability and vibration sensitivity are concerns for mechanically switched systems.

An alternative approach in development by the authors is electronic multiplexing according to the scheme shown in Fig. 3c and offers competitive or lower costs and likely more rugged construction than optical multiplexing while avoiding custom fiber switches. In this approach, the source and optical fiber related components are duplicated for each channel, but a single PMT is employed due to its high cost and significant size. One source at a time is electrically energized when a measurement for the corresponding sensor is desired. The emissions from all biosensors are continuously, simultaneously input into the large area PMT through a single filter, but only the optode receiving excitation light produces emission. LED sources are used to minimize the cost of these duplicated components. The rapid activation of LEDs allows electronic switching with sub-millisecond sampling intervals if desired. In order to reduce the use of LED source filters, which are significantly more expensive than the LEDs, a single inline filter can be used in parallel by multiple fibers.

4 Biosensors for Chemical Analytes

4.1 Hydrocarbons and Alcohols

Hydrocarbons are the most common group of contaminants found in soil and water, released through the activities associated with petroleum refining and fuel storage and transport. Alcohols are not normally considered to be environmental contaminants, but ethanol emissions from bakeries may be problematic. Furthermore, with the increased production and use of ethanol and perhaps butanol as biofuels, there is a greater chance that these chemicals will be released into the environment.

Two primary sensing concepts have been used to develop optical biosensors for hydrocarbons and alcohols: enzymatic oxidation with measurement of oxygen consumption, and induction-based detection. The enzyme-based biosensors have primarily been developed for ethanol and use the well known alcohol oxidase, which catalyzes the reaction of ethanol and oxygen to form acetaldehyde and hydrogen peroxide. While electrochemical transducers are frequently used in ethanol biosensors [8], some reports of optical transduction have been published. Mitsubayashi et al. described an optical biosensor in which alcohol oxidase was immobilized on the tip of a fiber optic oxygen sensor that used a photoluminescent ruthenium complex [9]. This biosensor was found to detect for ethanol in aqueous solutions in the range 0.5–9 mM and was also effective in gaseous samples with ethanol concentrations from 0.7 to 50 ppm. Wen et al. later described a similar system and achieved a lower detection limit in aqueous solution (30 μM), although their biosensor used an oxygen electrode [10]. Another group used coimmobilized alcohol oxidase and horseradish peroxidase, immobilized on an optical oxygen sensor, to measure methanol in *n*-hexane in the range 2–90 mM [11].

It would be of great interest to develop similar biosensors for the detection of hydrocarbons, and indeed enzymes exist that catalyze the reaction of oxygen with many different hydrocarbons. However, these mono- and dioxygenases also require

NAD(P)H as a cofactor, and thus can only be used in whole cells for short periods. A demonstration of this concept was described by Thavarungkul et al., who placed alginate-immobilized *Pseudomonas cepacia* cells in a flow cell near an oxygen electrode [12]. Detection of various aromatic compounds at approximately 1 mM was achieved. Rella et al. developed a more integrated system by immobilizing phenol-oxidizing *Bacillus stearothermophilus* in a hydroxyethyl methacrylate membrane that was coupled to an oxygen electrode [13]. Their biosensor responded to phenol, catechol, and related compounds, which is indicative of the moderate specificity of the oxygenase enzymes expressed by these cells. More recently, our group has demonstrated this concept as optical biosensors for the measurement of toluene using whole cells expressing toluene *o*-monooxygenase and an optical oxygen sensor. A detection limit of 0.3 mg L^{-1} was achieved, but the useful lifetime of the biosensors was very limited owing to the requirement for NADH [14].

Antibodies are commonly used in bioassay systems to accommodate the addition of reagents that are required in most sensing concepts to detect the binding of an antigen. Vo-Dinh et al. avoided this requirement by taking advantage of the natural fluorescence characteristics of benzo(*a*)pyrene [15]. Anti-BaP antibodies were immobilized on the tip of a biosensor and the binding of BaP was detected as fluorescence in response to excitation from a helium–cadmium laser. The system was very sensitive (LOD < 250 ng L^{-1}) with a specificity that depended on the antibody. However, the sensing concept is restricted to analytes that are photoluminescent

In addition to enzymes and antibodies, a third biosensing principle is that of induction. In this scenario, illustrated in Fig. 4, the analyte of interest induces transcription of an operon, typically by binding to a transcriptional activator that then binds to the promoter region, activating transcription [16, 17]. The transcriptional event is detected by fusing genes for a reporter protein behind the inducible promoter. While genes for a variety of reporter proteins have been used, those best suited for optical biosensors are those encoding autofluorescent proteins (often green fluorescent protein, *gfp*, but also red fluorescent protein, *dsred*), bacterial

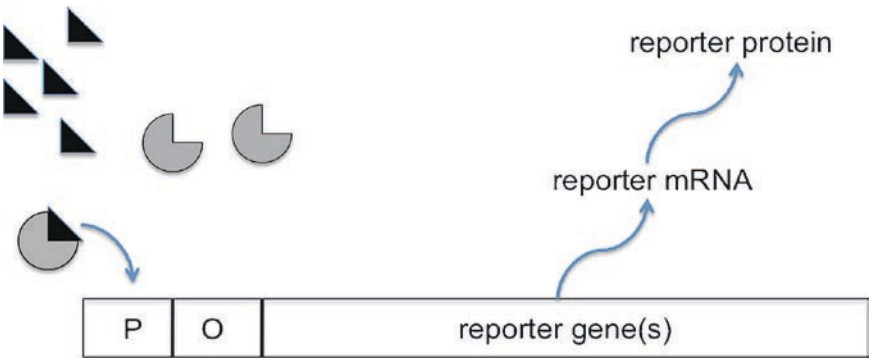

Fig. 4 Schematic depiction of induction biosensor concept. The analyte of interest (*triangle*) binds to a transcriptional activator and the complex activates transcription at the inducible promoter (P), leading to the synthesis of the reporter protein

luciferase (the *luxCDABE* cassette, when the enzymes for synthesis of the reaction substrate are included), and firefly luciferase (*lucFF*). Several excellent reviews are available on the use of these reporter proteins in biosensors and bioassays, including their relative advantages and disadvantages [16, 18]. The first report of the application of the induction biosensing approach came from the Sayler group, who constructed a biosensor for naphthalene and salicylate [19]. This biosensor was based on the reporter bacterium *Pseudomonas fluorescens* HK44, containing plasmid pUTK21 with the *luxDCABE* cassette fused with the *nahG* gene and under control of the P_{sal} promoter, which is regulated by the *nahR* gene product [20]. This reporter strain was immobilized onto the surface of a light guide, and the resulting biosensor produced light in response to both naphthalene and salicylate. Detection of 1.5 mg L^{-1} naphthalene was reported, and other measurements of real pollutant mixtures were obtained. However, it was necessary to supply the biosensors with growth medium to maintain the ability of the cells to perform the necessary transcription and translation, and the influence of the oxygen level and other parameters was noted. This induction-based biosensing concept has also been used to construct biosensors for toluene, styrene, benzene, and other chemicals (Table 1). While the sensing concept has the advantage of being very general (as long as the appropriate promoter and transcriptional activator can be identified), there are also the disadvantages of maintaining cell viability and of relatively low specificity. (Somewhat confusingly, the genetically modified cells are themselves often referred to as biosensors.) In addition to the application of induction-based biosensors for the determination of aqueous chemical concentrations, there have been reports of their use for assessing bioavailability enhancement from soils by surfactants [23, 32], illustrating the advantages of in situ measurements over the typical approach of laboratory-based methods involving organic solvent extraction.

4.2 Halogenated Organics

Halogenated organic chemicals form a diverse group of frequent interest for environmental monitoring. This group includes halogenated solvents (e.g., trichloroethene and tetrachloroethene) used in very large amounts each year, halogenated aromatics that are products or intermediates in industrial chemical processes (e.g., polychlorinated biphenyls (PCBs)), and halogenated aromatics and heterocyclic compounds that are formed as byproducts of combustion or chemical processing (e.g., dioxin). Many pesticides, such as lindane, are also halogenated.

Each of the three main optical biosensing strategies described in the previous section is also represented among the reports for biosensors for halogenated organics. The enzymatic approach has been described by several groups, most of which relied upon one or more enzymes from the class of hydrolytic haloalkane dehalogenases for detection of halogenated haloalkanes [33–37]. While most of these reports describe bioassays, and only one describes an optical biosensor, there is a common theme regarding the biocomponent. An optical biosensor for the measurement of 1,2-dichloroethane (DCA) in aqueous samples was published by our group [33]. This sensor

Table 1 Induction-based bioassays for chemical analytes

Analyte	Induction system	Reporter	Comments	Reference
Toluene and other aromatics	*xylR* and P_u of TOL plasmid of *Pseudomonas putida* mt-2	*lucFF*	Responds to xylenes and chlorinated toluenes more strongly than to toluene LOD of 10–20 μM toluene (in cell suspension) 2–3 h for full signal Linear response in the range 5–20 mg L^{-1} toluene Tested with contaminated ground water with good correlation to GC-MS values	[21]
Benzene, toluene, ethylbenzene, xylenes	*xylR* and P_u of TOL plasmid of *Pseudomonas putida* mt-2	*gfp, luxCDABE*	Responds to xylenes and naphthalene more strongly than toluene Luminescence reporter more sensitive but GFP signal more stable with time 2–3 h for full signal Low linearity of response Tested with contaminated ground water but low correlation with GC-MS values	[22]
Phenanthrene	*phnS* putative promoter/operator region (*Burkholderia* sp. strain RP037)	*gfp*	Responds to naphthalene more strongly than phenanthrene; also responds to anthracene 2 h for full signal LOD not determined	[23]
Styrene	P_{sty} of *Pseudomonas* sp. strain Y2	*lacZ*	Responds to styrene, toluene, and epoxystyrene 1 h for full signal LOD not determined	[24]
2-Haloacids	2-Haloacid dehalogenase promoter of *Pseudomonas* sp. strain 113	*luxCDABE*	Responds to propionic acid and 2-chloro- and 2-bromopropinic acid; otherwise, high specificity Linear response over range 0.5–2 mg L^{-1} chloropropionate but high signal variability 6 h for full signal	[25]
Chlorinated phenols	P_{dmp} and mutated DmpR from the *dmp* operon of *Pseudomonas* sp. strain CF600	*lacZ*	Responds to phenol and mono- and di-chloro-, methyl, and nitrophenols Response depends on DmpR mutant used Detection of 13 μg L^{-1} 2-chlorophenol	[26]

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	Dioxin-responsive element (DRE)	*seap*	Uses murine hepatoma cells LOD = 100 fM TCDD Responded to other DRE activators	[27]
Mercury	Promoter of *merR* of transposon 21	*luxCDABE*	LOD = 100 ng L^{-1} Hg^{2+} (scintillation counter) 1 h for full signal Detection in spiked natural waters correlated well to standard methods Selectivity not determined	[28]
Cadmium, lead	Promoter of *cad* of *Staphylococcus aureus* plasmid pI258	*lucFF*	Most sensitive to cadmium but also responded to antimony, zinc, tin, and lead. 2 h for measured signal LOD = 370 ng L^{-1} Cd	[29]
Uranium	Promoter of *urcA* of *Caulobacter crescentus*	*gfp*	LOD = 120 μg L^{-1} uranium Good selectivity with low sensitivity for lead, cadmium, and chromium. 3 h for full signal	[30]
Genotoxic compounds	Promoter of *cda* of *Escherichia coli*	*gfp*	LOD = 24 μg L^{-1} N-methyl-N-nitro-N-nitrosoguanidine (MNNG) Responds to MNNG, mitomycin C, nalidixic acid, others Linear response range for MNNG	[31]

Reporters: *lucFF* = firefly luciferase; *luxCDABE* = bacterial luciferase; *gfp* = green fluorescent protein; *lacZ* = β-galactosidase; *seap* = secreted alkaline phosphatase
LOD = limit of detection

design is based on a two-layer detection system, in which a pH-sensitive fluorophore [38] is immobilized on the tip of an optical fiber and whole cells are immobilized on the fluorophore layer. The cells in this biosensor contain the hydrolytic haloalkane dehalogenase DhlA, which adds water to a molecule of DCA, forming an alcohol, a chloride ion, and a proton. Protons from this dehalogenation reaction are detected as a pH change at the end of the optical fiber, and thus as a change in the fluorescence intensity. The change in fluorescence was shown to be directly proportional to the DCA concentration, with detection limits that are below 1 ng L^{-1} [39].

Using a similar principle with other dehalogenating enzymes, our group has developed biosensors for atrazine, lindane, and chlorohexane. The combination of enzymatic detection with photoluminescent transduction has also been used by our group to develop biosensors for chemicals for which the enzyme has not been characterized. Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo-dioxathiepin-3-oxide) is a cyclodiene organochlorine currently used as an insecticide. Recently, a *Pandoraea* sp. was isolated [40] with the ability to biodegrade endosulfan with a concomitant decrease in pH. This organism was combined with the optoelectronic transduction scheme of Campbell et al. [33] to produce an biosensor capable of detecting 2 μg L^{-1} endosulfan (Fig. 5). This compares favorably with laboratory-based liquid chromatographic methods that have detection limits of about 1 mg L^{-1}.

Antibody-based biosensing of chlorinated organics is difficult to achieve owing to the need for reagents in most detection schemes. Zhao et al. [41] reported on a competitive immunoassay format for PCBs in which anti-PCB antibodies were immobilized to an optical fiber. The fiber was initially loaded with a fluorescein-conjugated PCB analog, which was displaced by PCBs, leading to a decrease in

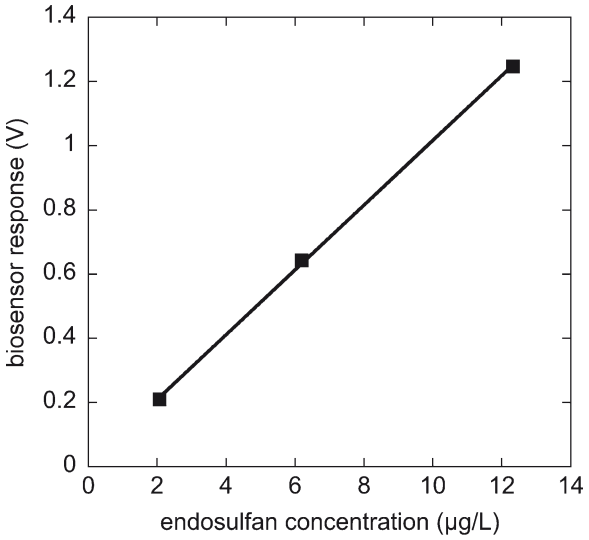

Fig. 5 Response of a *Pandoraea* sp.-based enzymatic biosensor to endosulfan in water

measured fluorescence. The response time was short (seconds to minutes), and thus it is possible that this assay could be implemented in a way that provides near real-time measurements.

Induction-based biosensing has been reported for halogenated organic acids, chlorophenols, chlorocatechols, and dioxins, among others (Table 1). Of particular note is a study by Kasai et al. [27] on the use of a nonbacterial host for the inducible gene. These researchers used a murine hepatoma cell line to host a plasmid containing a dioxin-responsive element fused to a viral promoter upstream of a reporter gene. In an assay mode, the reported limit of detection was 100 fM of dioxin. Although the reporter protein used in their study was not one that could be used with a photoluminescence transducer, it would be straightforward to make this conversion and create an optical biosensor from this sensing construct (albeit with the usual limits for induction biosensors of maintaining cell viability).

4.3 Nitro-, Phospho-, Sulfo-, and Other Substituted Organics

Many different substituted aromatic and aliphatic organic chemicals are of importance for environmental monitoring. For example, organophosphates have been used as pesticides (e.g., methyl parathion) and as nerve agents (sarin). Sulfonated organics are used in the dye industry (e.g., methylene blue). Organic molecules containing nitrogen moieties are also used in the dye industry (e.g., Acid Orange 7), as explosives (e.g., trinitrotoluene), and as precursors for organic syntheses (e.g., nitrobenzene for aniline). Despite the very large scale at which these chemicals are used (and the significant toxicity of many of them), there are relatively few reports of biosensors developed for their detection, even when bioassays are also considered.

In principle, an optical biosensor for a particular substituted organic could be developed using an enzyme, antibody, or induction sensing scheme. In the case of an enzyme-based biosensor, it would be necessary to find an enzyme that catalyzes a reaction with the analyte to produce or consume a species that could be detected optically, either directly or indirectly. One example is the use of organophosphate hydrolase (OPH), which hydrolyzes organophosphates to produce protons and cleavage products, some of which are chromophoric (e.g., *p*-nitrophenol from parathions). The group of Mulchandani and Chen has generated a variety of biosensor designs based on OPH, two of which can be adapted for photoluminescence-based biosensing. The first is the detection of the protons produced in the reaction. Originally, this was done by immobilizing OPH or OPH-expressing bacteria on a pH electrode [42]; our group has subsequently used a pH optode to produce an optical biosensor for paraoxon with a detection limit below 1 ng L^{-1} (unpublished results). The second OPH-based biosensor concept involves the use of aerobic microorganisms that consume the products of the OPH reaction and measurement of the corresponding decrease in oxygen. Although the Mulchandani and Chen group used oxygen electrodes to demonstrate this concept [43, 44], an oxygen optode could also be used to create a photoluminescence-based biosensor.

As noted previously, antibody-based optical detection methods for substituted organics nearly always rely on the addition of a reagent to detect the antibody–antigen binding and are thus assays instead of biosensors. Semicontinuous measurements could be performed if the detection system is incorporated into a flow injection analysis system [45], although the ability to obtain in situ measurements would still be limited. Some reports of antibody-based photoluminescence assays have been published. For example, an assay for trinitrotoluene (TNT) was developed based on the competition between TNT in the sample and fluorophore-labeled trinitrobenzene that was added [46]. The two nitroaromatics competed for antibody immobilized on an optical fiber, with higher TNT concentrations corresponding to lower fluorescence measurements. A detection limit for TNT of 10 μg L^{-1} was obtained.

The development of induction-based biosensors for substituted organics depends upon the ability to identify transcription regulatory elements that are responsive to the analyte of interest. Two approaches to this challenge have been reported. In one case, DNA microarrays were used to identify genes in *Saccharomyces cerevisiae* that were induced in the presence of the organophosphate paraoxon as well as those associated with the hydrolysis of paraoxon [47]. The promoters of highly responsive genes were fused to genes encoding either red (yDSRed) or green (yEGFP) fluorescent protein to create yeast strains that could be used as biocomponents in photoluminescence-based biosensors. The second approach was to use mutation-prone PCR and DNA shuffling to create variants of the toluene-responsive XylR regulator that were instead responsive to mono-, di-, and tri-nitrotoluenes [48]. These two reports demonstrate that the desired responsive elements can be identified through genomic searches or through modification of known regulators.

4.4 Metals and Other Inorganics

Although organic chemicals are the most common environmental contaminants, the detection of inorganic compounds is also of importance. Heavy metal contamination through metal plating operations (mainly chromate) and from acid mine drainage (e.g., copper, cadmium, mercury) are of concern in many areas around the world. Arsenic and selenium are found in high levels in lakes and groundwater as a result of natural and human factors. Furthermore, nitrate and phosphate contamination of waters may occur through over-application of fertilizers or as runoff from cattle feedlot operations.

Enzyme-based biosensors for such inorganics are limited by the ability to identify reactions with reactants or products that are detectable with a photoluminescence scheme, and antibodies that are specific for such small molecules are difficult to obtain. Thus, nearly all reports of biosensors for this group of contaminants rely on the induction approach. This is facilitated by a large body of research on mechanisms of metal resistance in microorganisms, which has revealed numerous metal-responsive genetic elements [49–51]. Many such biosensor constructs have been developed for mercury. For example, Selifonova et al. [28] used portions of

the mercury resistance operon of transposon 21 fused to the *luxCDABE* cassette to create *E. coli* reporter strains for Hg^{2+}. A scintillation counter was used to detect luminescence and detection as low as 1 nM was reported. Harkins et al. [52] compared nine such Hg^{2+} reporter strains constructed with different regulatory elements, reporter proteins, and host organisms. Similar bacterial reporter strains have been developed for cadmium [29, 53], lead [54], copper [55], uranium [30], nickel [56], arsenic [57, 58], and other metals (Table 1).

An interesting binding-based biosensor for copper was developed by Zeng et al. [59]. Carbonic anhydrase was labeled with a fluorophore in such a way that the binding of Cu^{2+} to the protein resulted in changes in fluorescence intensity and lifetime. Detection of 0.1 pM Cu^{2+} in seawater was reported, and the authors note that similar sensors for other cations could be developed using variants of carbonic anhydrase.

4.5 Chemical Mixtures

In the environment, contaminants are most often found in mixtures. This applies to soil and ground water at hazardous waste sites, as well as waste waters from industrial and municipal sources. Common examples of chemical mixtures that often become pollutants include gasoline and other petroleum fuels, pesticides, and wood-treating substances. Landfill leachates are complex mixtures that contaminate ground water supplies around the world. Pollutant mixtures may contain only organic chemicals or may also include inorganics, heavy metals, or radionuclides. The presence of mixtures of analytes poses at least two challenges for environmental measurements. The first is the ability of an analytical method to detect a specific chemical of interest in a background of other chemicals, some of which may have similar structures. This is the issue of selectivity and is an important characteristic of any analytical method. The second challenge for analysis of chemical mixtures is the goal of obtaining information on the entire mixture, the chemical composition of which is normally not known. In this section, we consider biosensor approaches to this problem of general mixture analysis.

The traditional methods used to characterize chemical mixtures in wastewaters are chemical oxygen demand and biological oxygen demand (BOD). The latter method is based on measurement of the oxygen consumed in a certain period by a microbial culture growing on organic chemicals in the sample. Numerous groups have adapted this approach to construct optical BOD sensors. These sensors involve a microbial culture immobilized on an oxygen optode. Thus, Chee et al. [60] used a pure culture of *Pseudomonas putida* to construct an optical BOD biosensor. When calibrated with an artificial wastewater of known composition, this biosensor yielded measurements that correlated well with the standard method over a low BOD range (1–10 mg L^{-1} BOD). The response time was 15 min, providing a considerable throughput advantage over the standard 5-day measurement time. The choice of microbial culture is important for any BOD analysis since a wide range of organic chemicals may be present, and thus other groups have evaluated

mixed cultures. For example, Jia et al. [61] used a coculture of *Trichosporon cutaneum* and *Bacillus subtilis*, Xin et al. [62] used three species, and Kwok et al. [63] used activated sludge. All of these biosensors performed well on the tested samples, but their efficacy for a wider range of sample types was not established.

A different approach to the assessment of chemical mixtures is to evaluate their toxicity. The older methods for accomplishing this in the environmental toxicology field (e.g., Daphnia acute toxicity assay, Ames mutagen assay) cannot be adapted to a biosensor format. A newer method, the Microtox assay (Strategic Diagnostics Inc., Newark, DE, USA) is based upon the decrease of the luminescence emitted by the bacterium *Vibrio fisherii* in the presence of toxins (and is thus a measure of acute toxicity). This assay has been modified to a continuous flow-through system [64] and could in principle, be converted to an optical biosensor mode (although no such reports were found). Although useful and able to report additive toxicity effects within a mixture, these assays are subject to false positive responses from anything that decreases metabolic activity.

While the Microtox-type assays provide a measure of acute toxicity, a range of induction-based biosensors have been described for the assessment of genotoxicity, oxidative stress, radiation, and other forms of toxicity [65–67]. In these biosensors, a regulatory element is identified that is associated with the particular type of toxicity. This element is then fused to a reporter gene (e.g., *luxCDABE*) to create a reporter construct as outlined above. The first of these toxicity reporter strains was described by Van Dyk et al. [68] and involved the fusion of a heat shock promoter (*dnaK* or *grpE*) to the *lux* cassette. Reporter strains for genotoxicity have been based on the promoters *recA* [69] and *cda* [31] in *E. coli*, and *RAD54* in *S. cerevisiae* [70]. Oxidative stress from superoxides has been detected using the promoters for *zwf* and *fpr* in *E. coli* [71] and general oxidative stress was reported by a fusion with the promoter of the *pgi* gene in *E. coli* [72]. Lee et al. [73] extended this concept by developing an array of twelve oxidative stress-responsive reporter strains. A different array concept with 20 reporter strains was described by the same group for the detection of oxidative toxicity (promoters for *soxS* and *sodA*), genotoxicity (promoters for *recA* and *gltA*), and membrane damage (promoters for *grpE* and *fabA*) [74]. In all of these reports, changes in luminescence were measured using laboratory luminometers and cells were cultured on agar plates or in liquid culture (vials or microwell plates). One of the few reports on the implementation of these assays toward biosensor formats described a flow system with separate chambers for cell cultivation and sample testing [74].

5 Summary and Future Developments

As the examples summarized above illustrate, optical biosensors with photoluminescence as the transduction mechanism have been developed for many different analytes of environmental interest. The use of enzymes as the biocomponent is prevalent and is perhaps the oldest form of environmental optical biosensor (particularly

if one considers that the Clark oxygen electrode used in the earliest examples can readily be replaced with a ruthenium-based oxygen optode). Enzyme-based optical biosensors often have the advantage that the enzymatic biocomponent does not require a cofactor and thus inactive cells or even purified enzymes can be used. This simplifies storage requirements and normally results in longer lifetimes than other types of biosensors. Induction-based reporter constructs are well suited for optical biosensing because the most common reporter proteins are either luminescent (e.g., luciferase) or fluorescent (e.g., GFP). However, since this detection mechanism relies on transcription and translation, the reporter strains must be maintained in a growth state prior to and during the measurement. There is also some question as to whether the signal is reversible – i.e., whether the biosensor/assay can be reused. Finally, although antibody-based biosensors have many excellent attributes, they are not well matched with transduction by photoluminescence owing to the need to add reagents.

As access to fresh water supplies becomes more limited, the need for environmental biosensors will increase. For example, direct reuse of waste water is already planned, and the protection of public safety will be best served by continuous monitoring of the effluent from these treatment systems. Furthermore, some ground and surface water contaminants are now seen as possible food contaminants (e.g., pesticides) and thus a new application area for these biosensors is emerging. Biosensors have excellent potential to fill these needs because they offer the ability for high-throughput and specific detection, particularly in comparison with most chemosensors (which are not usually specific) and chromatography (not high throughput). The specificity of biosensors can be tuned by using different biocomponents (directed DNA evolution is an exciting approach for this), and biosensor arrays offer the possibility of determining particular chemical concentrations within a mixture.

However, in order for biosensors to find commercial use, several key challenges must be met:

1. Biosensors must be validated in a range of real sample matrices and their sensitivity to temperature, pH, oxygen concentration, and other factors established.
2. Biosensors must be evaluated in chemical mixtures, particularly those containing molecules similar to the analyte of interest. Biosensor arrays hold considerable promise for measurements in mixtures.
3. The development of optoelectronic hardware for photoluminescence-based biosensing must not be overlooked. New optical configurations, optical components, and electronic circuits can improve the sensor's performance. If field portability is a goal, designs specific for that setting must be used and issues of changing ambient conditions addressed.

Acknowledgments Development of the ideas in this essay and several of the results cited herein were supported by US National Science Foundation (Grant BES-0529048), the Environmental Security Technology Certification Program of the Department of Defense (Contract CLU-0015), the Colorado Agricultural Experiment Station (COL 00643), and a NATO Collaborative Linkage Grant (EST. CLG 980504). Anne Glindkamp and Victor Acha obtained the endosulfan biosensor results in the Reardon laboratory.

References

1. Fähnrich K, Pravda M, Guilbault GG (2003) Biosens Bioelectron 18(1):73
2. Salmain M, Fischer-Durand N, Pradier CM (2008) Anal Biochem 373(1):61
3. Dennison MJ, Turner APF (1995) Biotechnol Adv 13(1):1
4. Rogers KR (1995) Biosens Bioelectron 10(6/7):533
5. Pieper S, Mestas S, Lear K, Zhong Z, Reardon K (2008) Appl Phys Lett 92(8):081915
6. Agishev R, Gross B, Moshary F, Gilerson A, Ahmed S (2006) Optic Laser Eng 44(8):779
7. Hecht H, Kolling M (2001) Sens Actuators B Chem 81(1):128
8. Azevedo AM, Prazeres DMF, Cabral JMS, Fonseca LP (2005) Biosens Bioelectron 21(2):235
9. Mitsubayashi K, Matsunaga H, Nishio G, Toda S, Nakanishi Y (2005) Biosens Bioelectron 20(8):1573
10. Wen G, Zhang Y, Shuang S, Dong C, Choi M (2007) Biosens Bioelectron 23(1):121
11. Wu X, Choi M, Chen C, Wu X (2007) Biosens Bioelectron 22(7):1337
12. Thavarungkul P, Hakanson H, Mattiasson B (1991) Anal Chim Acta 249(1):17
13. Rella R, Ferrara D, Barison G, Doretti L, Lora S (1996) Biotechnol Appl Biochem 24:83
14. Zhong Z, Fritzsche M, Pieper S, Wood TK, Lear KL, Dandy DS, Reardon KF (2008)
15. Vo-Dinh T, Tromberg BJ, Griffin GD, Ambrose KR, Sepaniak MJ, Gardenhire EM (1987) Appl Spectrosc 41(5):735
16. Harms H, Wells MC, van der Meer JR (2006) Appl Microbiol Biotechnol 70(3):273
17. Marques S, Arandaolmedo I, Ramos J (2006) Curr Opin Biotechnol 17(1):50
18. Paitan Y, Biran D, Biran I, Shechter N, Babai R, Rishpon J, Ron EZ (2003) Biotechnol Adv 22(1/2):27
19. Heitzer A, Malachowsky K, Thonnard JE, Bienkowski PR, White DC, Sayler GS (1994) Appl Environ Microbiol 60(5):1487
20. Burlage RS, Sayler GS, Larimer F (1990) J Bacteriol 172(9):4749
21. Willardson BM, Wilkins JF, Rand TA, Schupp JM, Hill KK, Keim P, Jackson PJ (1998) Appl Environ Microbiol 64(3):1006
22. Li Y, Li F, Ho C, Liao VH (2008) Environ Pollut 152(1):123
23. Tecon R, Wells M, van der Meer JR (2006) Environ Microbiol 8(4):697
24. Alonso S, Navarro-Llorens JM, Tormo A, Perera J (2003) J Biotechnol 102(3):301
25. Tauber M, Rosen R, Belkin S (2001) Talanta 55(5):959
26. Wise AA, Kuske CR (2000) Appl Environ Microbiol 66(1):163
27. Kasai A, Hiramatsu N, Meng Y, Yao J, Takeda M, Maeda S, Kitamura M (2004) Anal Biochem 335(1):73
28. Selifonova O, Burlage R, Barkay T (1993) Appl Environ Microbiol 59(9):3083
29. Tauriainen S, Karp M, Chang W, Virta M (1998) Biosens Bioelectron 13(9):931
30. Hillson N, Hu P, Andersen G, Shapiro L (2007) Appl Environ Microbiol 73(23):7615
31. Norman A, Hansen LH, Sorensen SJ (2005) Appl Environ Microbiol 71(5):2338
32. Keane A, Lau PCK, Ghoshal S (2008) Biotechnol Bioeng 99(1):86
33. Campbell DW, Müller C, Reardon KF (2006) Biotechnol Lett 28(12):883
34. Henrysson T, Mattiasson B (1993) Biodegradation 4(2):101
35. Hutter W, Peter J, Swoboda H, Hampel W, Rosenberg E, Kramer D, Kellner R (1995) Anal Chim Acta 306(2/3):237
36. Peter J, Hutter W, Stöllnberger W, Hampel W (1996) Biosens Bioelectron 11(12):1215
37. Peter J, Hutter W, Stöllnberger W, Karner F, Hampel W (1997) Anal Chem 69:2077
38. Zhujun Z, Zhang Y, Wangbai M, Russell R, Shakhsher ZM, Grant CL, Seitz WR, Sundberg DC (1989) Anal Chem 61(3):202
39. Acha V, Damborsky J, Reardon KF (2008)
40. Siddique T, Okeke BC, Arshad M, Frankenberger WT (2003) J Agric Food Chem 51(27):8015
41. Zhao CQ, Anis NA, Rogers KR, Kline RH, Wright J, Eldefrawi AT, Eldefrawi ME (1995) J Agric Food Chem 43(8):2308

42. Mulchandani A, Mulchandani P, Chen W (1998) Field Anal Chem Technol 2(6):363–367
43. Lei Y, Mulchandani P, Chen W, Mulchandani A (2006) Sensors 6(4):466
44. Mulchandani P, Chen W, Mulchandani A (2006) Anal Chim Acta 568(1–2):217
45. Ruzicka J, Hansen EH (1988) Flow injection analysis, 2nd edn. Wiley, Weinheim, pp 528
46. Shriver-Lake L, Breslin KA, Charles PT, Conrad DW, Golden JP, Ligler FS (1995) Anal Chem 67(14):2431
47. Schofield D, Westwater C, Barth J, Dinovo A (2007) Appl Microbiol Biotechnol 76(6):1383
48. Garmendia J, De Las Heras A, Galvão T, De Lorenzo V (2008) Microb Biotech 1(3):236
49. Collard JM, Corbisier P, Diels L, Dong Q, Jeanthon C, Mergeay M, Taghavi S, van der Lelie D, Wilmotte A, Wuertz S (1994) FEMS Microbiol Rev 14(4):405
50. Silver S (1996) Gene 179(1):9
51. Verma N, Singh M (2005) BioMetals 18(2):121
52. Harkins M, Porter AJ, Paton GI (2004) J Appl Microbiol 97(6):1192
53. Park J, Sohn M, Oh D, Kwon O, Rhee S, Hur C, Lee S, Gellissen G, Kang H (2007) Appl Environ Microbiol 73(19):5990
54. Ivask A, Francois M, Kahru A, Dubourguier HC, Virta M, Douay F (2004) Chemosphere 55(2):147
55. Stoyanov JV, Magnani D, Solioz M (2003) FEBS Lett 546(2/3):391
56. Tibazarwa C, Corbisier P, Mench M, Bossus A, Solda P, Mergeay M, Wyns L, van der Lelie D (2001) Environ Pollut 113(1):19
57. Ramanathan S, Shi W, Rosen BP, Daunert S (1997) Anal Chem 69(16):3380
58. Tauriainen S, Karp M, Chang W, Virta M (1997) Appl Environ Microbiol 60:1414–1420
59. Zeng HH, Thompson RB, Maliwal BP, Fones GR, Moffett JW, Fierke CA (2003) Anal Chem 75(24):6807
60. Chee GJ, Nomura Y, Ikebukuro K, Karube I (2000) Biosens Bioelectron 15(7/8):371
61. Jia JB, Tang MY, Chen X, Li Q, Dong SJ (2003) Biosens Bioelectron 18(8):1023
62. Xin LL, Wang XD, Guo GM, Wang XR, Chen X (2007) Meas Sci Technol 18(9):2878
63. Kwok NY, Dong SJ, Lo WH, Wong KY (2005) Sens Actuators B Chem 110(2):289
64. Stolper P, Fabel S, Weller M, Knopp D, Niessner R (2008) Anal Bioanal Chem 390(4):1181
65. Gu M, Mitchell R, Kim B (2004) Adv Biochem Eng Biotechnol 87:269
66. Sørensen SJ, Burmølle M, Hansen LH (2006) Curr Opin Biotechnol 17(1):11
67. Vollmer AC, Van Dyk TK (2004) Adv Microb Physiol 49:131
68. Van Dyk TK, Majarian WR, Konstantinov KB, Young RM, Dhurjati PS, LaRossa RA (1994) Appl Environ Microbiol 60(5):1414
69. Bartolome AJ, Ulber R, Scheper T, Sagi E, Belkin S (2003) Sens Actuators B Chem 89(1/2):27
70. Knight AW, Goddard NJ, Fielden PR, Barker MG, Billinton N, Walmsley RM (1999) Meas Sci Technol 10(3):211
71. Niazi JH, Kim BC, Gu MB (2007) Appl Microbiol Biotechnol 74(6):1276
72. Niazi J, Kim B, Ahn JM, Gu M (2008) Biosens Bioelectron 24(4):670
73. Lee J, Youn C, Kim B, Gu M (2007) Biosens Bioelectron 22(9/10):2223
74. Lee J, Mitchell R, Kim B, Cullen D, Gu M (2005) Biosens Bioelectron 21(3):500